JAN 19 1929

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LXXXVII—No. 3

Issued January 1, 1929

BALTIMORE, U. S. A.

1929

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

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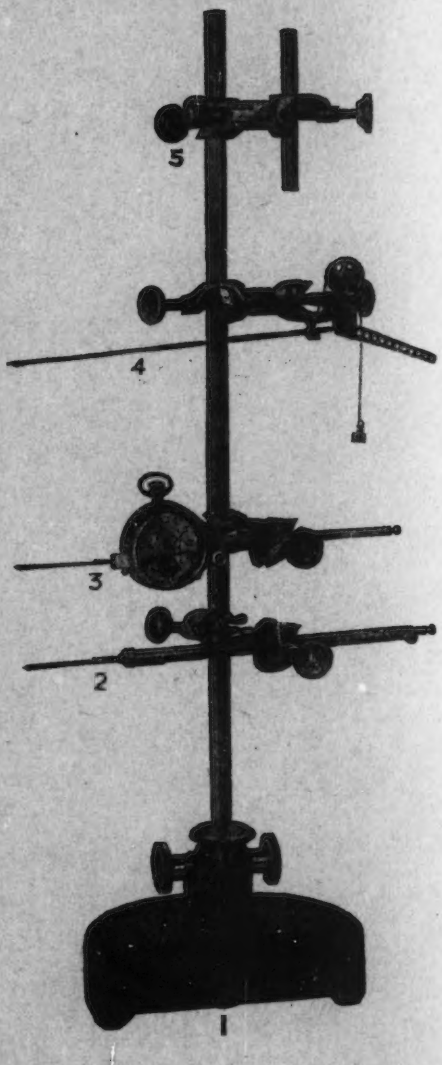

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 87 JANUARY 1, 1929 No. 3

THE EFFECT OF THE INTERVAL FOLLOWING DECEREBRATION ON THE RESULTS OF LOW SPINAL TRANSECTION

A. FORBES AND P. C. BAIRD, JR.

From the Laboratories of Physiology of the Harvard Medical School

Received for publication November 7, 1928

The temporary modification of reflexes below the level of a spinal transection is commonly designated "spinal shock." In the older researches reflex response was almost invariably evoked by tetanizing currents or other prolonged stimuli, and showed depression following spinal transection. The introduction of the single stimulus permitted closer analysis of the effects of transection and led to the suggestion that "spinal shock," insofar as it affects the flexion reflex, might consist merely in the abolition of after-discharge or central "reverberation" in the various reflex paths involved (Forbes, 1922; Forbes, Cobb and Cattell, 1923). This suggestion was based on the observations of Sherrington and Sowton (1915) and of Forbes, Cobb and Cattell (1923). Sherrington and Sowton found that in the decerebrate cat the flexion reflex, as evoked by a single induction shock, showed mechanically an increase immediately following spinal transection. Forbes, Cobb and Cattell observed that the electric response of the motor nerve in the flexion reflex showed a similar immediate increase, that on repetition of the stimulus the decline of subsequent electric responses became less, and there was less accumulation of muscular contraction as recorded with an approximately isotonic lever of small inertia.

McCouch (1924) has criticized this interpretation of spinal shock on the ground that transection of the spinal cord in the decerebrate animal is quite different from that in the intact animal, since in the former case the path from the cerebral cortex to the spinal centers has already been interrupted. He speaks specifically of "an interval between decerebration and spinal transection sufficient for a considerable degree of recovery from the shock due to cutting cerebral flexion arcs."

In order to test the influence of the interval elapsing between decerebration and spinal transection upon the effect of the latter on the flexion

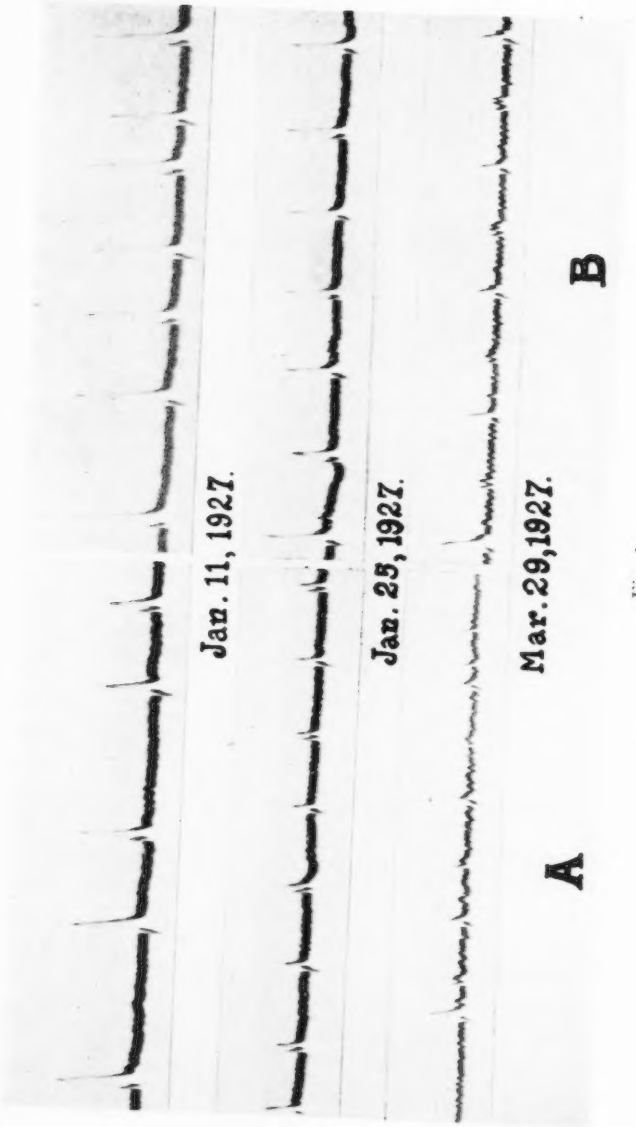

Fig. 1

reflex, the following experiments were undertaken. The discussion of the significance of these experiments, and of other theoretical considerations raised by McCouch, will be deferred to a subsequent paper dealing with the effects of spinal transection on the crossed extension reflex.

METHOD. The procedure employed was essentially the same as that described by Forbes, Cobb and Cattell (1923). Briefly it consisted in decerebrating the animal under deep anesthesia, stimulating the popliteal nerve with single break shocks or with a series of stimuli repeated at intervals of from 4 to 10 per second, recording the monophasic electric responses from the peroneal nerve with a Hindle string galvanometer, aided by an electron-tube amplifier, then transecting the spinal cord at the level of the last rib and repeating the procedure to determine whether there was any change in the size of response.

In the series of experiments reported by Forbes, Cobb and Cattell, brevity of the interval between decerebration and spinal transection was not an object; therefore the preparation for spinal transection and the dissection of the peripheral nerves were performed after decerebration. In the present experiments it was desired to make the interval between decerebration and spinal transection as short as possible; therefore as soon as the animal was etherized, laminectomy was performed and the cord exposed. Then the popliteal and peroneal nerves were dissected out and cut peripherally, stimulating electrodes were secured in contact with the former and the latter was made ready to be led into the moist chamber containing leading-off electrodes connected with the galvanometer. The animal was then decerebrated. In order to provide an adequate base line, i.e., an adequate series of records made before spinal transection, for purposes of comparison, it was deemed advisable to record responses for

Fig. 1. Monophasic responses in motor nerve with series of reflex stimuli, before and after spinal transection. Electron-tube amplification in all; electric artefacts, showing time of stimuli, appear as alternately descending and ascending excursions (make and break shocks) about 8σ before responses.

In all records string diameter, 2.5μ; resistance, 18,700 ohms; tension, 95.5 m. per amp.; magnification, 490.

DATE	DECEREBRATION TO TRANSECTION (INTERVAL IN MINUTES)	TIME OF OBSERVATION WITH RESPECT TO TRANSECTION		SPEED OF FILM
		A, minutes before transection	B, minutes after transection	
				cm. per sec.
January 11, 1927	55	1.5	5.5	11.0
January 25, 1927	38	4.0	2.5	10.3
March 29, 1927	26	1.5	3.0	10.1

a period of from 10 to 20 minutes before transecting the cord. The interval between decerebration and spinal transection varied in the different experiments from 26 to 55 minutes. In the experiments of Forbes, Cobb and Cattell, the interval varied from 2 to 6 hours. The earliest interval after spinal transection at which we were able to take a record was one minute.

In applying a series of repeated stimuli a rotary interrupter in the primary circuit was sometimes used.

RESULTS. Ten experiments were performed in which records were obtained both before and after spinal transection. In two of these the animal became moribund and died approximately ten minutes after spinal transection; the responses in this moribund condition cannot be considered fair examples of the normal response after transection. In the eight remaining experiments large numbers of responses were recorded after

TABLE 1

DATE	DECEREBRATION TO TRANSECTION (INTERVAL IN MINUTES)	AVERAGE EXCURSION (INITIAL RESPONSE)	
		Before transection	After transection
		mm.	*mm.*
January 11, 1927	55	13.5	18.2
January 25, 1927	38	4.4	8.9
March 29, 1927	26	5.0	6.8
October 17, 1928	52.5	0.6	1.4

transection, and in each case the series both before and after was sufficient to warrant a definite statement of the effect.

Even at the briefest interval (26 minutes) spinal transection was followed by essentially the same changes in the character of response as those described by Forbes, Cobb and Cattell. The size of the electric response to a single stimulus was greatly increased, and there was less decline in the size of the response following the first in a repetitive series, 4 to 10 per second. Figure 1 shows typical examples of responses to repeated stimuli, before and after spinal transection, in three experiments. The size of the response to a single maximal stimulus may be judged from the first of each series, since there was a rest of several seconds to permit recovery from fatigue or "equilibration" (Gerard and Forbes, 1928) preceding each series of stimuli. These effects were found in every experiment.

Comparing these experiments with the previous series, we find that whether spinal transection was performed less than an hour after decerebration, or more than two hours after, the size of the single response shows an immediate increase, and this increase is of about the same order of

magnitude. The relative increase in those experiments which were devoid of all sources of confusion is shown in table 1.

In the present experiments, as in those of Forbes, Cobb and Cattell, the relative decline of successive responses in a series was less after transection than before. Certain effects were observed in these experiments which were not specifically noted in the earlier series. Responses recorded within a minute after spinal transection were already larger than those obtained before, but during the next three or four minutes there was a further increase in size of response. An interesting difference between the behavior of the initial response and the "equilibration" values during the first few minutes after transection appeared in some experiments. Further observations on this point are in progress, and the description of the findings will be deferred till these have been completed.

The main conclusion to which the present experiments lead is that the characteristic effects following immediately on spinal transection in the decerebrate cat, viz., increased single response and lessened "equilibration" decline, occur whether transection is performed less than half an hour after decerebration or after a much longer interval. It follows that if these effects depend for their existence on an interval "sufficient for a considerable degree of recovery from the shock due to cutting cerebral flexion arcs," the required interval must be less than half an hour. We have not found it feasible to reduce the interval further.

SUMMARY

1. The suggestion that spinal shock, in its bearing on the flexion reflex, might consist in abolition of after-discharge, has been criticized by McCouch, who has postulated previous interruption of cerebral flexion arcs and a certain interval of recovery therefrom as a prerequisite to the observed immediate effects of low spinal transection, viz., increase in single electric response (motor nerve flexor muscle) and lessened decline in subsequent responses of a rapid series.

2. Experiments on spinal transection in the decerebrate cat have been repeated with the interval between decerebration and transection reduced to less than one hour and in two cases to 26 minutes.

3. Essentially the same results were obtained at the briefer intervals as at longer intervals. It is inferred that if an interval of recovery from interruption of the cerebral arcs is a prerequisite to the observed effects this interval must be less than half an hour.

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THE CARBON DIOXIDE CAPACITY OF THE HUMAN BODY AND THE PROGRESSIVE EFFECTS OF CARBON DIOXIDE UPON THE BREATHING

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Received for publication October 22, 1928

The quantitative relation between carbon dioxide tension and carbon dioxide absorption in the case of blood has long been known. In the bodily economy the absorption by the tissues must be at least as important as that by the blood. What is the carbon dioxide combining power of the whole body at equilibrium? How rapidly does carbon dioxide, when introduced into the alveoli of the lungs, arrive by way of the blood in the tissues? What, meanwhile, are the effects of carbon dioxide upon its own distribution through the agency of the particular tissues which regulate the breathing and the circulation?

Brocklehurst and Yandell Henderson (1927) attempted to measure the carbon dioxide capacity of man in the absence of equilibrium. They assumed that the time required for the passage of carbon dioxide from lung-alveoli to tissues was negligible, and deduced the carbon dioxide capacity from the rate of accumulation of carbon dioxide in a rebreathing system. That carbon dioxide penetrates tissues with great rapidity has often been noted, particularly as indicated by the well-known instances in which carbon dioxide permeates most exceptionally as compared with other acids. But when corrected for its high solubility and low molecular radius, its rate of penetration is no greater than that of other molecular substances.

It is apparent in numerous data of many investigators that the breathing of constant percentages of carbon dioxide influences the various circulatory and respiratory factors in man slowly and progressively. Then these factors in themselves may, on the one hand, delay the transport of carbon dioxide, or, on the other hand, may be direct indications of the rate at which the carbon dioxide penetrates into particular tissues (in this case the respiratory center, vasomotor neurones, or blood-vessel walls). Recently Padget (1928) has illustrated on man the oft-recognized fact that the breathing responds only with a considerable lag to increases in carbon dioxide tension.

We attempted in this investigation to measure the carbon dioxide retention when considerable percentages of carbon dioxide were breathed; and the extra carbon dioxide elimination when ordinary air was subsequently breathed. All the expired air was collected over a period of more than two hours, of which about half an hour was occupied in breathing a mixture of air and about five per cent of carbon dioxide. Knowing how much carbon dioxide was inspired, and how much carbon dioxide was expired, it remained to estimate the production of carbon dioxide in the body from the oxygen consumption and the equilibrium respiratory quotient.

PROCEDURE. We were able to undertake these experiments because two large spirometers of 520 and 330 liters capacity were available.[1] With these were assembled two smaller spirometers, each of 140 liters capacity. Inspiratory mixtures of carbon dioxide from a cylinder were made up with room air in both the two large spirometers; expired air was collected in rotation in the two smaller spirometers and in the 330-liter one. The expired air was thus collected in about twelve samples. As each collection was completed, the air was mixed, the dead space for sampling was flushed out, and a sample was withdrawn into a mercury collecting tube. In each sample both oxygen and carbon dioxide were analysed in duplicate, after the entire experiment was over. Two preliminary experiments were made without complete gas analyses, followed by three complete experiments.

Alveolar air was collected from the tubing just beyond the expiratory side of the mouth valves. Each sample followed a normal inspiration; it was drawn directly into a Haldane-Henderson gas analyser and was immediately determined for carbon dioxide. Samples were given by the subject as rapidly as analyses could be performed, so that from 20 to 35 were obtained during an experiment.

The volumes of air expired were measured not only at the end of each sample collected, but, with some interruptions, the position of the spirometer was taken at 30-second intervals. Meanwhile the rate of breathing was recorded by a chest pneumograph and tambour.

The subject sat on a low stool, leaning forward so that the gas analyser for alveolar samples could be situated directly under the mouth valves.

The dead spaces in all of the breathing apparatus were determined. The air spaces within the spirometers were measured by making up a mixture of carbon dioxide and air within the spirometer, sampling it; then pressing the mixture out to the zero point of the spirometer through the dead space; then, drawing in a measured amount of air and mixing it with the residual carbon dioxide, finally sampling the mixture. During an experiment, when each spirometer was emptied, obviously, air of known composition remained

[1] Our thanks are due to Prof. W. S. McCann and the Department of Medicine for the use of these two spirometers and of the laboratory space.

in the dead spaces. Thus the composition of the new air could be corrected for the composition and the quantity of the air which remained from the previous filling. In the majority of cases the corrections were not large, for the smaller spirometers, which were most used, had dead spaces of only 5 to 10 per cent of their volumes when filled. The capacities of the four spirometers were calibrated against each other.

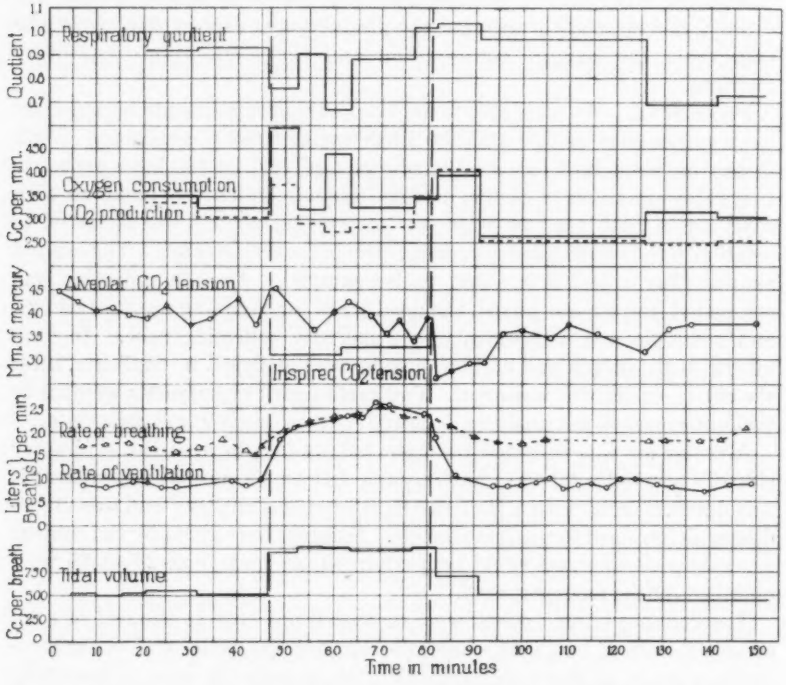

Fig. 1. Experiment 5. Subject N. Inspired 792 liters of 19.70 per cent oxygen plus 4.37 per cent carbon dioxide for 33.7 minutes, between the times marked by the vertical dashes. The total amount of carbon dioxide inspired, at N. T. P. dry, was 30.8 liters.

The burettes of the Haldane-Henderson gas analysers were calibrated. The expiratory volumes and tidal volumes were uncorrected for atmospheric conditions; but the total quantities of carbon dioxide and oxygen contained in each expiratory spirometer sample were reduced to standard temperature, pressure, and humidity.

RESULTS. 1. *Carbon dioxide equilibrium in the body.* Whether the subject attained equilibrium with the gas mixture inspired may be judged

by several criteria (figs. 1 and 2, table 1). The best criterion was the respiratory quotient, as used by Shaw (1926). In two of the three experiments the quotient came to have approximately normal values during the latter part of the breathing of carbon dioxide, and in all three experiments was normal after the extra carbon dioxide had been breathed off.

The second criterion of equilibrium was the constancy of the alveolar carbon dioxide tension. In all the experiments the alveolar carbon dioxide tension was constant to the normal degree after the extra gas had been breathed off. But in no case was the carbon dioxide tension constant

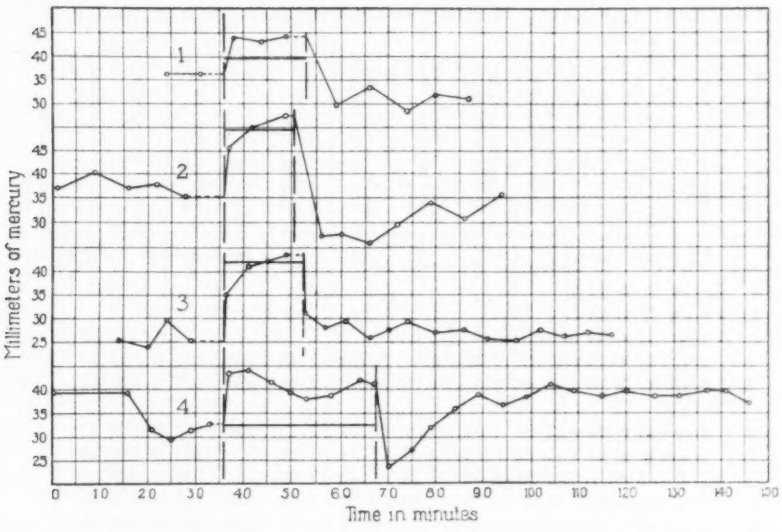

Fig. 2. Alveolar carbon dioxide tensions during experiments 1, 2, 3 and 4. The experiments are plotted so that the breathing of carbon dioxide started in each case at the abscissa of 36 minutes. The end of the breathing of carbon dioxide is in each experiment indicated by vertical dashes. The tension of carbon dioxide in the inspired air is indicated for each experiment by a heavy horizontal line.

for any length of time during the breathing of carbon dioxide. The reason for this fact can be seen in the records of total ventilation, for the breathing was never uniform but was faster and slower in periods. The ventilation curves were quite accurately the converse of the alveolar tension curves. The breathing was, therefore, never under a uniform stimulus. We deem it unlikely that a steady state can be attained within a half-hour of breathing a mixture of high carbon dioxide content.

The third criterion of equilibrium between body and inspired air was the uniformity of the rate of ventilation itself. In one or two instances the

alveolar tensions appeared to be constant while the total ventilation was still changing. Under the conditions of these experiments it was likely that any change in the quantity of air breathed indicated a change in respiratory stimulus and, therefore, a lack of carbon dioxide equilibrium in some tissues.

TABLE 1

Experiment 4, Subject N

Inspired 810 liters of 19.78 per cent oxygen plus 4.55 per cent carbon dioxide for 32.4 minutes, beginning 0.6 minute before the end of sample 5, and ending 0.2 minute before the end of sample 10. The total amount of carbon dioxide inspired, at N. T. P. dry, was 32.1 liters.

EXPIRED SAMPLE NUMBER	DURATION OF COLLECTION	ACTUAL VOLUME OF SAMPLE	VOLUME OF SAMPLE AT N.T.P. DRY	ACTUAL CONCENTRATION OF OXYGEN EXPIRED	ACTUAL CONCENTRATION OF CO_2 EXPIRED	CORRECTED CONCENTRATION OF OXYGEN UTILIZED*	CORRECTED CONCENTRATION OF CO_2 EXPIRED*	CONCENTRATION OF CO_2 EXPIRED MINUS CO_2 INSPIRED*	OXYGEN UTILIZED PER MINUTE	CO_2 PRODUCED PER MINUTE	RESPIRATORY QUOTIENT	AVERAGE CO_2 TENSION IN ALVEOLAR AIR	RESPIRATORY DEAD SPACE
	minutes	*liters*	*liters*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*cc.*	*cc.*		*mm. Hg*	*cc.*
1	5.28	31.9	27.9									39	
2	3.68	32.1	28.1										
3	5.90	55.8	48.9									39	
4	9.82	103.8	90.9	17.15	3.52	3.81	3.52	3.49	352	323	0.915	30	92
5	7.87	83.2	73.1	16.88	3.51	4.16	3.51	3.48	386	323	0.837	32	97
6	8.38	135.0	118.5	17.08	5.68	3.07	5.79	1.15	366	163	0.375	43	
7	4.53	111.0	97.7	17.54	5.63	2.44	5.74	1.23	526	265	0.504	40	
8	5.47	134.6	118.1	17.83	5.90	2.34	5.91	1.42	505	307	0.607	38	
9	9.85	321.8	280.2	18.47	5.19	1.78	5.30	0.70	505	199	0.393	40	
10	3.72	112.2	98.6	17.94	5.79	1.87	5.79	1.21	496	321	0.647	40	
11	7.25	134.0	117.6	18.00	3.66	2.77	3.54	3.51	450	570	1.266	25	−19
12	36.57	300.5	261.6	16.64	3.82	4.96	3.55	3.52	355	252	0.709	37	141
13	15.47	128.4	112.7	16.57	3.78	4.64	3.79	3.76	338	274	0.810	39	138
14	13.52	109.1	95.7	16.64	3.89	4.55	3.77	3.74	322	265	0.822	39	137
15	7.78	45.3	39.5	16.43	3.59	4.78	3.58	3.55	243	180	0.750	39	

*The corrections were for the air previously occupying the dead spaces of the breathing apparatus, and for the change in volume of the air between its inspiration and its expiration.

The last criterion of equilibrium was the dead space of the breathing. This was constant in the fore- and after-periods, but was never of a probable value during the breathing of carbon dioxide. This was, of course, a very searching criterion of accuracy, for it required proper measuring, sampling, and analysing of a sample of a hundred liters or more of expired air, as well as proper sampling and analysing of several samples of alveolar air. We

did not feel neglectful in not attaining to this height of experimental exactitude during the intake of carbon dioxide, for probably physiological as well as technical limitations were involved.

In the fore-period of each of our experiments there was some overbreathing while the subject was on the valves. This was most extensive in experiment 4. The smaller amounts of overbreathing which occurred in the other experiments were relatively negligible, because of the corresponding lack of

TABLE 2

Carbon dioxide capacities

SUBJECT	EXPERIMENT	DURATION ALLOWED	RESPIRATORY QUOTIENT USED	CO_2 DEFICIT	CO_2 TENSION DIFFERENCE IN MM. MERCURY	cc. OF CO_2 PER MM. TENSION DIFFERENCE
		minutes		*cc.*		
S. 73 kgm.	3, saturation	5.9	0.91	650	15	40
		16.3	0.91	790	19	40
	3, saturation	14.8	0.91	1,570	17	90
		26.3	0.91	2,080	16	130
	blood, calculated					20
N. 63 kgm.	4, saturation	8.4	0.82	1,610	9	180
		32.0	0.82	4,900	7	700
	4, desaturation	7.3	0.82	1,450	13	110
		43.8	0.82	0	0	
	blood, calculated					18
	5, saturation	6.1	0.89	390	4	100
		16.8	0.89	1,020	5	200
		30.3	0.89	1,030	2	510
	5, desaturation	14.1	0.85	940	9	100
		49.4	0.85	1,990	7	280

constancy in the alveolar tension of carbon dioxide during the subsequent periods of intake of carbon dioxide. Shaw (1926) induced overbreathing before administering carbon dioxide to cats, in order to make the change of carbon dioxide tension greater when this gas was administered. But so little is known of the rate at which equilibrium is attained with a changed tension of the gas in the body, that errors can be avoided only by starting from a long-continued basal condition.

2. *Carbon dioxide capacity.* At each saturation of the body up to a

higher level of carbon dioxide, the alveolar tension changed by 5 to 19 mm. of mercury (table 2). In the subsequent desaturations the tension changed by similar magnitudes, though transiently it was greater in three of the five experiments (fig. 1). The quantities of carbon dioxide held by or given up by the body in response to these changes of tension were between 400 and 2100 cc., with one exceptionally higher value. The results are listed in table 2, as computed on the basis of various durations of the saturation and desaturation processes. Actually there is little doubt that the longest periods for which the calculations were made are preferable, when judged by all the four criteria discussed above. Nevertheless, the errors accumulated rapidly with time, so that the results for the first periods of changed intake are also listed. These are the data most convincing that the direction of saturation and desaturation was in each experiment that to be expected, and of a remarkably uniform initial amount. But in the first periods absorption of carbon dioxide was in no case complete.

Average human blood is known to take up, in the neighborhood of 40 mm. tension, about 0.50 volume per cent of carbon dioxide for each millimeter change of tension. In one of our equilibrations the whole body changed its carbon dioxide content by only twice the blood's content; but in the five other instances the capacity was from 6 to 25 times that of the blood alone (table 2).

During the experiments no marked change occurred in the titratable acid concentration of the urine. Davies, Haldane and Kennaway (1920) found no measurable change in the carbon dioxide capacity of the blood during prolonged breathing of carbon dioxide.

The errors involved in measuring the carbon dioxide absorption of the body by this method were great, for 1 or 2 liters of this gas were being estimated not merely among some 40 liters of metabolised carbon dioxide, but among over 800 liters of inspired and expired air. The errors in the desaturation period were somewhat less, for here only about 13 liters of carbon dioxide were expired, and about 400 liters of air. With sampling and corrected analyses 2 per cent in error, and corrected volumes 1 per cent in error, the total possible experimental error amounted to nearly 100 per cent of the final quantities obtained. It is evident that only by a large series of experiments could accurate values be obtained with this method, for the probable error of the result diminishes from this initial value of 50 per cent only as the square root of the number of measurements.

The carbon dioxide capacities indicated in these experiments were considerably greater than those indicated by the method of Brocklehurst and Henderson (1927) on man. They agreed in magnitude with the values of Shaw (1926) which were obtained upon anesthetized cats. They agreed also with some values which have been obtained by Doctor Fenn (unpublished) by direct analysis of muscular tissue excised from cats.

Analogous evidence of the distribution of considerable acid to the tissues is furnished by the experiments of Van Slyke and Cullen (1917). When they slowly injected sulfuric acid into the veins of a dog, they found, after an hour and more, a depression in the carbon dioxide combining power of the blood corresponding to only one-sixth of the injected quantity. The remainder either went into other tissues or drew alkali from them. Palmer and Van Slyke (1917) noted that ingested sodium bicarbonate raised the carbon dioxide combining power of the plasma by an amount as though the bicarbonate were equally distributed throughout 70 per cent of the body. Banus and Katz (1927) showed that when blood containing hydrochloric acid was perfused through the hind-leg of a dog its carbon dioxide combining power was partially restored. Fenn (1928) found that the carbon dioxide dissociation curves of isolated muscles and nerves of frogs were similar in slope to that of blood.

The carbon dioxide capacity of the body must be particularly significant in the measurement of respiratory metabolism, for every millimeter change in alveolar tension causes the retention or excretion of 100 cc. of carbon dioxide and therefore a tremendous shift in the apparent respiratory quotient.

3. *Hyperpnea.* When carbon dioxide was inspired, the breathing, of course, augmented. This augmentation had the effect of maintaining an alveolar and tissue tension of carbon dioxide relatively near the normal level. In one of us (subject S) the extra ventilation was attained almost wholly by increasing the depth of breathing until the tidal volume was 3600 cc. In the other (subject N) the rate and the depth of breathing increased by proportionate increments.

There was a considerable lag in the increase of ventilation when the inspired air contained less than 5.5 per cent of carbon dioxide. The alveolar tension at first was, therefore, very high; but as the hyperpnea increased, the alveolar tension was gradually reduced. After the carbon dioxide intake ceased, the hyperpnea persisted for some minutes, with the result that the alveolar tension of carbon dioxide decreased considerably below normal. Later the respiratory quotient was also below normal, indicating a retention of carbon dioxide while the alveolar tension was being restored.

The effect of these two lags in respiratory response was in both cases to hasten the exchange of carbon dioxide between air and tissues, by accentuating the tension difference between tissues and alveolar air.

There was, however, an irregular character to the breathing. Whether this indicated shifts in the circulatory as well as in the respiratory response was not clear. The 40 minutes required for the adjustment to normal carbon dioxide tension after the intake of carbon dioxide ceased, indicated that some of the changes in response to carbon dioxide were remarkably slow. It was probable that the responses were quicker during carbon diox-

ide intake than after it, since not only was ventilation then increased, but also the rate of the circulaton (Goldstein and Dubois, 1927).

The fact that extra oxygen consumption is required for the effort of hyperpnea has often been recognized. In the three experiments here reported it amounted to −7, 36 and 17 per cent respectively of the basal consumption. At the rate of 5.5 cc. of oxygen for each extra liter of air respired, a figure assumed by Schneider, Truesdell and Clarke (1924), the values would be 22, 25 and 22 per cent respectively. The oxygen tension in the alveoli was relatively uniform throughout. There was no evidence of a considerable change in the resting metabolism with the acidosis of carbon dioxide.

4. *The time factor.* When the tension of carbon dioxide inspired was higher than the tension in the normal alveolar air, the absorption of carbon dioxide by the body seemed to be sufficiently great actually to keep the alveolar tension below the tension inspired for some minutes. In experiments 2 and 3 (fig. 2) this period lasted five minutes, and something like 100 liters of air was ventilated in the alveoli, which alone must account for the absorption of at least one liter of carbon dioxide. But these two experiments were not conclusive because the tension of carbon dioxide was lower in the alveolar air than in the expired air throughout the entire 15 minutes of carbon dioxide inspiration.

When desaturation began, the carbon dioxide was given off from the body very rapidly at first. Thus in experiment 5 (fig. 1), although air was breathed for only the last 1.2 minutes of a 5.2-minute expiratory sample, this period caused the respiratory quotient to rise far above normal, and about one fourth of all the extra carbon dioxide breathed off during desaturation was given up in this period.

That a long time is required to attain equilibrium with inspired carbon dioxide mixtures has not been generally appreciated. Attempts to correlate changes in respiration with the progress of rebreathing experiments are largely complicated by the time factor. In Shaw's experiments it required 90 minutes to saturate an anesthetized cat with carbon dioxide, using artificial breathing. We do not feel confident that in the 33 minutes of hyperpneic breathing of carbon dioxide in our experiments saturation was complete. Attempts to measure the circulation rate, vascular reactions, capillary responses, and the like, during carbon dioxide inspiration or during forced breathing must take the time factor into account.

It is probable that the circulation is the limiting factor in the velocity of equilibration of carbon dioxide between tissues and inspired air. The velocity is, therefore, a measure of the "diffusing capacity" of the blood system. Whether the changes induced by carbon dioxide are all due to the progressive penetration of the carbon dioxide into the tissues, or whether there are, in addition, physiological changes in the tissues of the kind known as adaptations, is unknown.

We are indebted to Mr. A. Petrilli for working with us in the experiments and in the tedious calculations, and for drawing the charts.

SUMMARY

1. When the human body was saturated or desaturated to a new level of carbon dioxide tension, carbon dioxide was retained or given up by the body amounting to between 40 and 700 cc. for each millimeter change in alveolar carbon dioxide tension. In five instances out of six, therefore, 3 to 18 per cent was absorbed by the blood, and 82 to 97 per cent of the carbon dioxide was absorbed by the other tissues.

2. During a half-hour of breathing 4.5 per cent of carbon dioxide, no steady state was reached; but ventilation, alveolar tension, respiratory quotient, and dead space varied, sometimes periodically. At the close of the carbon dioxide breathing, half an hour or more was required for the normal respiratory equilibrium to return.

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SOME OBSERVATIONS ON THE SYMPATHETIC INNERVATION TO THE SKELETAL MUSCLES OF GOATS

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Received for publication October 22, 1928

In considering and reconsidering, first, the report by Royle (1924) that in decerebrate goats tone is diminished or absent in a limb deprived of sympathetic innervation, and, secondly, the failure of numerous other workers to confirm this finding using the more common laboratory animals (cat and dog), two sources of discrepancy have seemed possible. On the one hand, the worker: some detail of experimental technique, some mode or bias of observation might be at fault. On the other hand, the animal: the mechanism for the maintenance of tone may differ essentially either in structure or in manner of operation in the goat and in the cat and dog.

Much recent work, including some by the authors, Hines (1926) and Hines and Tower (1928), has emphasized that even from muscle to muscle within an animal as well as from animal to animal throughout the vertebrate series the morphology of the innervation of each component of skeletal muscle may vary remarkably. Thus it seemed increasingly plausible that a peculiarity of innervation of goat's skeletal muscle, perhaps as described by Hunter and Latham (1925), might be the basis of Royle's almost uniquely positive results.

At the same time it was noted that in the bulk of work designed to test the Hunter-Royle theory of tonic innervation, except, perhaps, on man, Royle's operation probably was not repeated as described. Subsequent workers, striving to improve, resected rather than avulsed, the sympathetic supply to the region selected. This very technical perfection, by minimizing injury done to the spinal nerves, seemed not inadequate to account for the failure to detect any deficiency in muscular function. Indeed, throughout the history of this perplexing subject, far antedating Hunter and Royle, there has been a seeming inverse proportion of positive findings to technical skill.

Mode of experimentation. The experiments designed to check the two sources of discrepancy just suggested comprise studies both of function and of morphology. The former alone are reported here. The results of

[1] The Henry Strong Denison Medical Foundation, by a grant to one of the authors (S. S. T.), contributed largely to this research.

histological examination of the material will be issued later. Five young goats were used. In each of these the sympathetic innervation to one fore leg was interrupted by one of two operations. Based on published directions and the vivid recollection by one of the authors (S. S. T.) of Royle's performance on a man, the operation of cervical sympathetic ramisection was executed according to Royle's technique in two animals of the series. In the remaining three, the cervical sympathetic chain (inferior, middle and superior cervical ganglia with the intervening cord) was resected entire, the inferior ganglion through the vertebral end of the first intercostal space according to the technique devised by Tower (1926); the middle and superior ganglia and cord through a midline incision in the neck. All wounds healed rapidly.

The animals were examined in ether rigidity,—first, during recovery from surgical anesthesia, again, before decerebration, and a pair a fortnight after operation.

The use of the legs in standing, walking, running, and jumping was carefully studied, both immediately on recovering from the anesthetic and each week thereafter.

Notes were kept on the condition of the eyes and on the temperature of the ears. (Estimation of the temperature of the skin over the fore legs was unsatisfactory because of the hair.) In the two animals operated on by Royle's technique there was no evidence of cervical sympathetic lesion in the eyes and no vasodilatation in the ears. The three animals subjected to unilateral cervical sympathetic resection, on the contrary, developed the complete Horner's syndrome on the side of lesion,—narrowing of the palpebral fissure, conjunctival vasodilatation, and slight pupillary constriction. The blood vessels of the ear were also relaxed. The eye condition persisted, somewhat mitigated. The vasodilatation largely disappeared in the first week, although thereafter the vessels took no part in general vasodilatation, during exercise, for example, or under ether.

Lastly, the animals were decerebrated. After ligating both carotid arteries, the left half of the cranial vault was removed, the brain stem transected at the level of the superior colliculus, the parts of the brain anterior to the cut removed, and, after bleeding had ceased, the cavity loosely packed with dry cotton. For at least the first hour after transection artificial respiration was administered (compressed air through a tracheal T tube). Four goats, two operated upon by each technique, were successfuly decerebrated, one of each pair thirty days, and the other, sixty days after operation. It was thought that by the end of one month vascular readjustment would be complete, yet no question of regeneration could arise. Two months were allowed to elapse between operation and decerebration to satisfy adequately Royle's requirement of time for the maximum effect of sympathectomy to develop.

OBSERVATIONS. Although the results of hours of observation could be summarized in one statement—that in no examination made on any goat, irrespective of the type of operation previously employed, were differences detected between the partially denervated and the normal fore limbs in tone or use greater than appeared from time to time in these functions in the entirely normal hind limbs of the same animal—yet for completeness of the record the observations will be detailed.

The use of the limbs in standing, walking, running and jumping was studied extensively immediately on the animal's recovery from surgical anesthesia and weekly thereafter. In order to expose and resect the lower cervical ganglion in one of the goats the first thoracic nerve had had to be retracted. For two weeks this animal dragged the hoof on the side of lesion slightly. Other than this, none of the animals used the operated fore leg in any way differently from the normal either immediately after the operation or two months after interruption of the sympathetic innervation to the part. The body weight appeared to rest equally on the fore legs. The operated leg showed no tendency to give way under the strain of long standing or in jumping from a table. Seemingly even the trauma incident to operation produced no discomfort, certainly no dysfunction.

Normal tone, that present in the muscles under ordinary conditions, was repeatedly examined in all the goats: initially, on recovery from the operative anesthesia; finally, just previous to decerebration, and at intervals between. For this examination, the animals were placed back down in a V shaped animal board, so constructed to keep the spine and head in alignment. Now when quiet, passive extension of the fore legs followed by pressure exerted on the hoofs commonly excited the animal to maintain the extension after the observer's hand was removed for a variable period of time—a few seconds, a minute, sometimes many minutes. Neither the normal nor the partially denervated leg in any of the animals tended more readily or more completely to assume or to maintain longer the posture thus passively imposed. Figures 1 to 4 illustrate this reaction in two goats operated on, one by each technique, fourteen days previously. The resistance opposed at elbow and shoulder to passive flexion was slight but also bilaterally equal. Passive extension was maintained by the hind legs, too, and cursory observation brought out here the same inconsistent variability seen in the fore legs.

The posture of the fore legs was examined under other conditions as, for example, when the animal was held suspended free of the floor by the ears or back. Again no asymmetry in posture, no inequality in the resistance offered to displacement was discernible.

Ether rigidity is the extensor rigidity occurring in a certain stage of light anesthesia. Very probably it is somewhat equivalent to decerebrate rigidity in representing activity of lower motor centers relieved of cortical

control. This condition was examined during recovery from the surgical anesthesia; again, thoroughly, previous to decerebration; and in a pair of animals, for two hours on the fourteenth post-operative day. The method of examination was as just outlined for the unanesthetized animals. The maintenance of spontaneous or of passively imposed extension and the resistance opposed to passive flexion were repeatedly tested in the two fore legs, and incidentally, also in the hind legs. None of these examinations discovered more than transitory and inconsistent asymmetry, not even immediately after operation in the presence of fresh trauma. Figures 5 to 8 were made during the examination of ether rigidity on the fourteenth day after operation. The two animals represent again the types of operation.

Under ether the goats exhibited a reaction which was met again later in decerebrate rigidity, thus supporting the belief that the tone examined in the two conditions is similar. The usually smooth execution of spontaneous extension or of relaxation from extension spontaneously or passively assumed was broken into a series of steps fairly regularly spaced at intervals of ten to twenty seconds. Relaxation, for example, progressed until, seemingly, there was sufficient disturbance of the relations within the muscles to excite a proprioceptive reflex checking the process. This fixed the position of the limb for a few seconds, after which relaxation proceeded. In spontaneous extension the appearance of a series of superimposed muscle jerks was produced. Outwardly the resemblance to the described behavior of so-called "plastic tone" was striking. Operated and normal fore legs exhibited this reaction indiscriminately.

Decerebrate rigidity was the condition most intensively studied in these experiments. Four goats, two of each type of operation, were successively decerebrated, one of each pair thirty days and the other sixty days after operation. The examination, requiring two or three hours, was conducted largely as described for the examination of the unanesthetized animals, that is, with the animal supine in the V board. This was supplemented by observations made when the animal was supported by a sling in the normal posture with feet on the table.

The degree of rigidity developed differed from animal to animal and varied from time to time in the same animal. It became greater as the examination progressed and was markedly increased by slight asphyxiation. On the whole it seemed less than described by Royle for at no time would a strong man have had difficulty in overcoming it. However, when the animal was placed in the upright posture, if the legs were carefully placed and the head prevented from falling to one side, it sufficed to maintain the animal standing free of the sling for many minutes. The joints most affected were the shoulder and elbow, and correspondingly, the hip and knee. The ankle, indeed, was often completely extended, but rarely

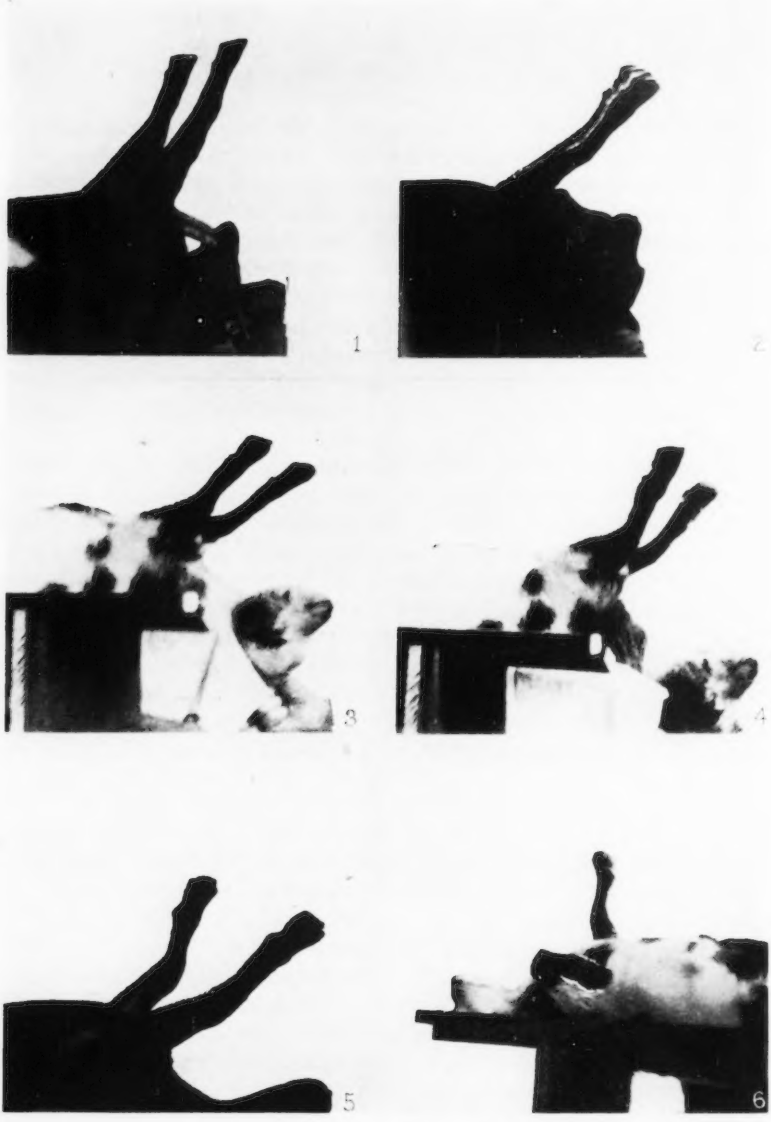

Fig. 1. Goat I. Right cervical sympathetic resection fourteen days previously. Passive extension assumed.

Fig. 2. Same. Fifteen minutes later. Passive extension still maintained.

Fig. 3. Goat II. Right Royle operation fourteen days previously. Passive extension assumed.

Fig. 4. Same. Four and one-half minutes later. Passive extension still maintained.

Fig. 5. Goat I. Right cervical sympathetic resection fourteen days previously. Ether rigidity.

Fig. 6. Goat II. Right Royle operation fourteen days previously. Ether rigidity.

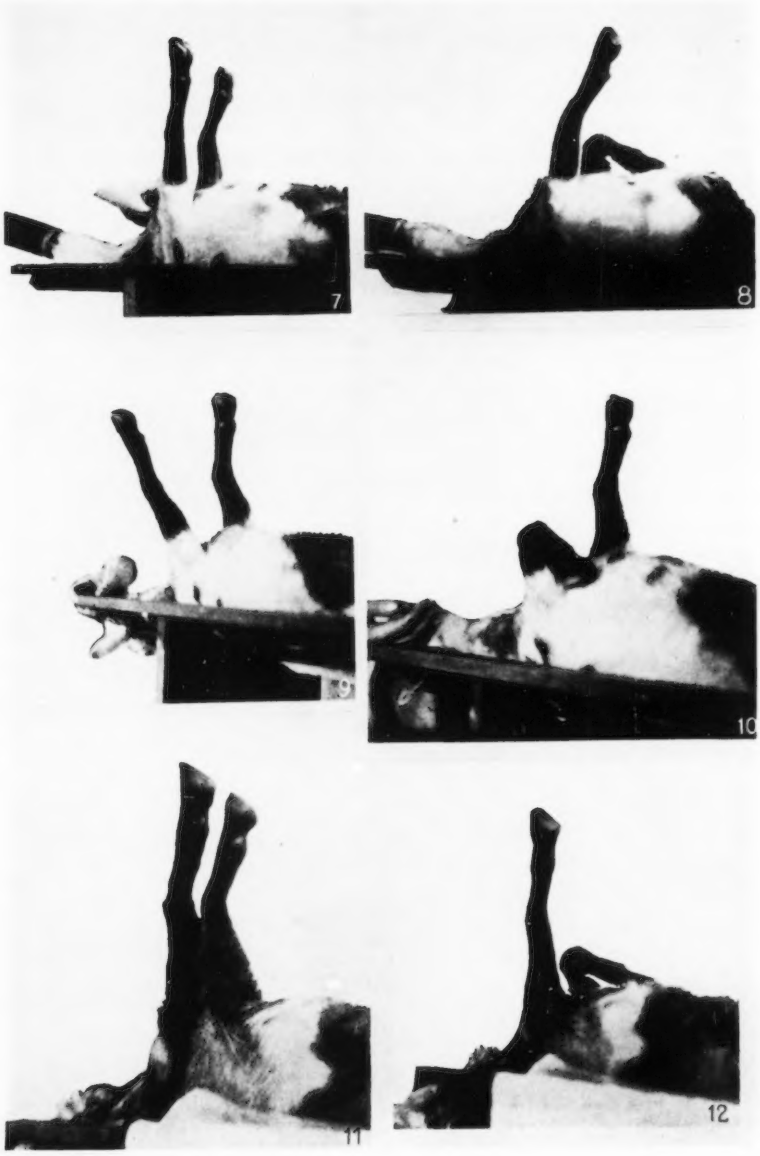

Fig. 7. Same. Few minutes later.

Fig. 8. Same. Later.

Fig. 9. Goat II. Right Royle operation thirty days previously. Decerebration 2:30 p.m. Picture 2:45 p.m.

Fig. 10. Same 2:55 p.m.

Fig. 11. Goat IV. Right cervical sympathetic resected 30 days previously. Decerebration 1:30 p.m. Picture 2:45 p.m.

Fig. 12. Same. 2:48 p.m.

offered more than slight resistance to passive flexion, and always in relaxation it was affected first.

Decerebrate rigidity, likewise, brought out no consistent difference between the normal and the partially denervated fore limb. Extensor tone developed with equal rapidity and completeness either spontaneously or in response to passive extension in the two fore legs, and its persistence suggested no disturbance of a "holding mechanism." The resistance offered to passive flexion was symmetrical. Such a phenomenon as the step extension and step relaxation, shown here identically as in light anesthesia, seemed to indicate the presence of "plastic tone," in both fore legs. In the animals operated by Royle's technique equally with those subjected to complete unilateral cervical sympathetic resection no deficiency in muscular function could be detected. Furthermore the interval elapsing between operation and decerebration, whether thirty or sixty days, in no wise modified the result. To be sure, from time to time the fore legs were not equally extended or relaxation of spontaneously or passively imposed extension was not concomitant, but these inequalities were no greater than were demonstrated from time to time in the entirely normal hind limbs of the same animal and appeared with no consistency on either side.

Figures 9 to 12 demonstrate the equal extension but variable maintenance of extension in the pair of goats which were decerebrated thirty days after operation. Figures 9 and 10 reproduce Royle's results in a goat operated by his technique. Here equal extension in the two fore legs was followed over a period of ten minutes by relaxation of the rigidity in the operated but not in the normal leg. This, however, was an incident in the examination in which frequently quite the contrary was seen. Figures 11 and 12 illustrate the contrary in an animal whose entire right cervical sympathetic chain was gone. Now equal extension was followed during the next five minutes by relaxation in the normal fore leg alone.

Reactions of the type so thoroughly studied by Magnus and de Kleijn (1912, 1915) demonstrating the influence of head and neck position on postural tone were easily elicited in these decerebrate goats. In the goats, as in cats, flexing the neck diminished the extensor tone of the fore limbs and, conversely, extending the neck augmented it. Rotating the head sharply to one side gradually increased the tone in the fore leg of that side while decreasing it contralaterally. The influence on the hind legs was less marked. These effects were studied with the animal supine and again in standing posture. As these reactions are par excellence functional adjustments of postural tone, their equal symmetrical exhibition by both fore legs in the presence of unilateral sympathectomy and irrespective of the type of operation used to produce this, seems to demonstrate beyond doubt that the sympathetic innervation has no essential rôle in the maintenance of postural tone.

All of the decerebrate animals exhibited at some time rhythmic movements of progression. These occurred usually late in the examination and best when the animal was in the upright posture. They could be excited by slight asphyxiation, by over inflating the lungs, or by noxious stimulation such as pinching between the toes. In one of the goats (III) these developed into running movements very well coördinated in all four legs. At no time was it noted that these appeared freer or less coördinated in one fore leg than in the other.

The muscles of these animals were inspected and palpated from time to time to detect gross atrophy. No decrease or increase of bulk on the operated side was noted; no change in resistance to palpation. The question of atrophy will be examined fully in the morphological studies. No trophic disturbance affected the skin, hair or hoofs. There was no increased susceptibility to infection. The last goat used (V) was kept for about three months. Toward the end of this time he lost weight and became weak. After an effort such as running around the room or jumping from a table to the floor his muscles exhibited a coarse tremor. The emaciation, weakness and tremor, however, affected all four extremities equally. The elimination of sympathetic innervation to one limb, even with the consequent vasomotor disturbance, did not grossly affect the trophic state of the part.

Discussion. The existence of a sympathetic innervation of skeletal muscle, its possible functions, are problems of long standing. The voluminous literature has been repeatedly digested, most ably by Cobb (1925); less thoroughly, previously, by one of the authors (Tower, 1926). There is no need to repeat. Inasmuch, however, as the data here presented challenge Royle's observations (1924, 1927) in part, it were well, perhaps, to restate his position. Hunter and Royle published in 1924 the results of studies made on goats, birds and on patients suffering from a variety of spastic conditions. In these the sympathetic innervation to one or more extremities was interrupted. The work on goats specifically interests us. Royle has recently (1927) recapitulated and amplified his original observations. These are, in short, that goats from which the left lumbar sympathetic chain has been removed may limp for several weeks but quickly recover normal walking and standing posture. When turned on the back, however, though both hind legs commonly extend, the operated leg soon becomes flexed and abducted more than the normal. The knee jerks "markedly differ in character," the relaxation time for the normal exceeding that for the operated leg, the entire movement in the latter appearing quicker. The resistance to passive flexion and extension is greater on the normal side. Under light anesthesia these differences are exaggerated. In decerebrated preparations they are maximal. In these there is a loss of resistance to passive motion in the operated limb.

The knee jerks differ as noted. Lengthening and shortening reactions (considered a criterion of the condition of plastic tone) are wanting in the operated leg or diminished. And extensor rigidity, though assumed, is not well maintained. The longer the interval elapsing between operation and decerebration the greater the differences to be noted, but these are, none the less, present immediately after operation. The bulk of the partially denervated muscles is, if anything, increased; certainly there is no atrophy. Regretting that the numerous observers who failed to confirm his results had not used his animal, the goat, Royle states that he has repeated his experiments in cats and reproduced repeatedly and consistently the results seen in goats, although, because of its small size and the consequent lesser effectiveness of gravity, the cat is an unsuitable animal.

The experiments on cats may be dismissed as briefly as given. Since De Boer's report in 1913 the workers using this animal, Cobb (1918), Kanavel, Pollock and Davis (1925), Hinsey and Ranson (1926), Coman (1926), using various methods of attack have uniformly failed to discover any function for a sympathetic innervation of skeletal muscle.

In a series of six sympathectomized dogs, Kuntz and Kerper (1926) detected a slight post-operative hypotonia, merely temporary,—a phenomenon also observed in a single animal by Foix (1924). On the other hand, both Meek and Crawford (1925) and Tower (1926), using far larger series of animals, failed to confirm this. Royle has not used dogs although in point of size suitable.

Coates and Tiegs (1928) have contributed most recently to the problem. Using dogs and goats, pigeons and fowl, they have repeated many of the classical experiments devised to test for sympathetic innervation. On goats and dogs, Royle's observations could not be confirmed. At most the muscles developed, months after operation, a seeming softness to palpation. Station and gait were normal. The knee jerk was unaltered. Kuntz and Kerper (1926) were not confirmed in the diminished resistance to be detected by tonus curves, following sympathectomy. Muscles deprived of sympathetic innervation were found, however, to fatigue more easily than normal muscles. No decerebrations were done. On birds, likewise, Hunter's observations were not confirmed. Sympathectomy modified wing posture not at all. After deafferentation the wing was tonic (as described by Trendelenburg) but the reactions interpreted by Hunter (1924) as shortening and lengthening, were found to be artifact.

The literature is, therefore, almost overwhelmingly in disagreement with Hunter and Royle. Their experiments have been repeated and amplified on a variety of animals, and, in part, on their chosen animals—goats and birds. Yet diminution of tone following sympathectomy remains almost uniquely their finding. However, those experiments on which Royle laid most emphasis were still directly unassailable; decerebrate rigidity had

not been reëxamined in goats. The studies presented in this paper provide this last desired evidence. The data fail to confirm Royle's report in any respect. Interrupting the sympathetic innervation to the one fore leg of a goat in no wise impairs the tone of that leg either as exhibited in decerebrate rigidity or in any of the other conditions examined.

The choice of animal cannot now be held responsible for the discrepancy between the findings of the earlier Australian workers and those of almost all others who have studied the sympathetic innervation to skeletal muscle. The conformity of data from this series of goats, two subjected to the operation of sympathetic ramisection devised by Royle for man, three to complete cervical sympathetic resection, eliminates the type of operation as a source for disagreement. The confusion in this problem must, therefore, be derived from the observer; some point in technical procedure, perhaps surgical, or in the mode or bias of observation must be at fault.

Inasmuch as a theory stands or falls on the validity of the evidence presented for its support it seems futile to discuss the elaborate concept of duality of tone and the mechanism postulated for its maintenance promulgated by Hunter. This has been repeatedly done, perhaps most thoroughly in the symposium of the Section on Neurology of the Royal Society of Medicine, 1926, participated in by Adrian, Elliot Smith, Walshe and others.

As to that elusive quality—tone—the observations recorded in this paper yield but one conclusion: that for the exhibition by goat's skeletal muscle of any variety of tonic activity now recognized, distinction of "plastic" and "contractile" quite unnecessary, an intact sympathetic innervation is not essential. Subtle moderating influences,—trophic, nutritional,—the sympathetic nervous system may later be shown to exert. But the final motor path of tone it does not contain. Tone, a participant in probably every variety of normal muscular activity, and seemingly not to be isolated as an entity, remains in skeletal muscle a function of the somatic innervation.

SUMMARY

One fore limb, in a series of five goats, was deprived of sympathetic innervation by one of two operations (by sympathetic ramisection after Royle's technique, or by complete cervical sympathetic resection). Normal posture and gait, and the hypertonus appearing under light ether anesthesia were then repeatedly examined during the next thirty or sixty days. Finally the animals were decerebrated. At no time were differences detected in posture, use, or tone of the fore legs exceeding the variation in the entirely normal hind legs of the same animal. Decerebrate rigidity developed simultaneously, and was equally maintained in the two fore legs. No atrophy or trophic disturbance was noted. The conclusion is drawn

that tonic acidity, studied as a component of total skeletal muscular function, is in no direct wise dependent on an intact sympathetic innervation of the muscle.

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THE ALLEGED PRESENCE OF CAROTIN IN PIG'S LIVER[1]

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Received for publication October 22, 1928

Underhill and Mendel (1) have described a dietary deficiency canine disease against which pig's liver is effective, likewise such carotin-containing foods as butter and carrots as well as crystallized carotin itself. According to their interpretation, "there is a direct relationship between the presence of carotinoids in the substances tested and the potent factor." Underhill and Mendel make no attempt to show that this relationship is a quantitative one. In fact the data presented support merely a qualitative relationship and this only in the case of butter and carrots. As for pig's liver, Underhill and Mendel state, "In considering carotin as the effective agent in this investigation an apparent contradiction may be found in the fact that fresh pig's liver contains the effective agent. According to Palmer, pig's liver does not contain carotin. On the other hand, this statement of Palmer's *must be*[2] erroneous for Prof. J. C. Drummond assures us from his own work that pig's liver does contain carotin. This apparent discrepancy is thereby clarified."

My (2) original statement regarding the absence of carotin from pig's liver is as follows. "A careful examination of pork liver showed no pigments to be present which were distinctly characteristic of carotin or xanthophyll although considerable difficulty was encountered in handling the material, due to the presence of bile and liver pigments." Underhill and Mendel have apparently overlooked the statement by Steenbock (3) in support of this. Referring to it Steenbock says, "Gross of this (Steenbock's) laboratory has obtained similar results (unpublished data). . . ." As for Drummond's assertions, he has never published any proof to support them. In a paper with Rosenheim (4) the statement is made that, "substances resembling the lipochromes do occur in pig's liver." In another place in the same paper it is stated that, "we have found in liver tissue besides carrotene and xanthophyll, a substance. . . .not identical" (with the lipochromes). This statement might be interpreted to indicate the presence of carotin in pig's liver, but the context refers to cod liver.

[1] Published with the approval of the Director as Paper No. 809 Journal Series, Minnesota Agricultural Experiment Station.

[2] The italics are mine.

The question of the presence of carotin in pig's liver seemed to me to be of sufficient importance to warrant its reinvestigation, particularly since I encountered certain difficulties in my earlier work, and also because Underhill and Mendel apparently regard the contradiction in their own work as settled by the private assurances of Professor Drummond.

I have been able to confirm in every particular the results secured more than ten years ago. In addition I am now able to show in what respects the carotin-like liver pigment is distinctly different from carotin. It seems evident to me that Drummond has drawn his conclusion because of the *partial* similarity to carotin of a pigment occurring in pig's liver in extremely small concentration. In discussing the occurrence of carotinoids in mammalian liver in my monograph (5) I pointed out that conclusions regarding the presence of carotin in liver based on the use of color reactions, solubility and phase tests are to be accepted with reserve until the spectroscopic and adsorption properties of the pigment are studied. None of the other properties are in reality specific for carotinoids.

Experimental. The separation of pigment for study as a carotinoid was carried out with two lots of pig's liver, each weighing three-fourths to one kilo. One lot was secured in March and the other in May, each time from a local retail market. The first lot was divided into two portions, which were treated differently, so that in reality the separation of pigment was made three times. The results were identical in each case. The third experiment only will be described because the spectroscopic properties of the pigment secured in this case were submitted to a quantitative as well as qualitative comparison with crystalline carotin from carrots.

The fresh liver was ground to a pulp and dried in an oven at 60°C. under 25 inches vacuum. The residue was ground to a fine flour in a Wiley mill. The powder was extracted by decantation with petroleum ether (b.p. 40–60°C.). The extract had a deep brownish red color. After the petroleum ether would take out no more color, the liver powder was dried again under vacuum and washed with ethyl ether until the extract was colorless. This extract had a reddish-yellow color, which lacked the brown shade shown by the petroleum ether extract.

The petroleum ether and ether extracts were kept separate. Each was evaporated to dryness at room temperature using the water pump for vacuum. Each residue was treated with sufficient colorless 15 per cent alcoholic KOH solution to saponify the glycerides present and refluxed for two and one-half hours in a boiling water bath. Each saponified solution was diluted with 3 volumes of H_2O, and extracted once with an equal volume of petroleum ether, followed by a similar extraction with ethyl ether. The two extracts were kept separate until after they were washed free from alkali, when they were combined and washed again until clear.

Two solutions were now on hand: 1, the non-saponifiable matter from the petroleum ether extract of the dried liver; 2, the same from the ethyl

ether extract which followed the petroleum ether washing. These two solutions behaved exactly alike during their preparation. Each had a faint yellow color which seemed to come entirely from the ether extract of the soap, the petroleum ether extracts apparently being colorless. The two solutions were now combined and represented the entire non-saponifiable matter from nearly 1 kilo of fresh pig's liver. The amount of coloring matter present may perhaps be judged by the fact that the color in 100 cc. volume was yellow, with a shade approximately half way between lemon and orange. If it were carotin the amount could not exceed one milligram.

The combined solutions were shaken with anhydrous Na_2SO_4, filtered, and taken to dryness under such vacuum as could be obtained with the water pump. The dry residue was dissolved[3] in warm methanol, the flask cooled in ice water, and the copious precipitate of sterols filtered off and washed with ice cold methanol. The filtrate was diluted with sufficient H_2O to reduce the alcohol concentration to 85 per cent. This caused a copious precipitation of yellowish, fatty-like substances, which could be filtered off after cooling the flask in ice water for one-half hour. The filtrate was clear and had a pale yellow color.

The color in this filtrate was submitted to the phase test. Petroleum ether extracted so little color from this solution that it was not visible until the alcohol layer was removed and the petroleum ether extract concentrated. When this was done, however, a golden yellow solution with a distinct brownish tint was obtained. On spectroscopic examination this solution showed only general absorption beyond the visible violet, whereas a pale yellow solution of carotin showed the characteristic absorption bands in the blue. The color of the liver pigment was distinctly different from carotin in the same solvent.

The yellow salvy precipitate which formed when the methanol was diluted with water was also examined for the presence of carotin on the supposition that the carotin content of the methanol might have been so high that the dilution caused the precipitation of most of it, the solubility of carotin in dilute alcohol being low. This salvy material dissolved readily in petroleum ether, giving a solution with the same golden yellow color and brown tint noticed in concentration of the petroleum ether extract of the dilute methanol. There was every reason to believe that this was the major portion of the same pigment, and was so considered. The petroleum ether was removed under suction, and the residue taken up at once with absolute ethyl alcohol in which it was readily soluble. The color was distinctly different from an alcoholic solution of carotin from carrots. A qualitative examination of its spectroscopic properties showed no bands

[3] A small amount of brown material would not dissolve on repeated heating with methanol. It readily dissolved in CS_2 but the solution showed no spectroscopic absorption bands, only general absorption of the violet.

and only general absorption beginning in the visible violet. This was true even for concentrations having a deep orange color.

Figure 1 shows a quantitative comparison[4] of the spectral transmittancy of the liver pigment and an absolute alcohol-petroleum ether solution of pure crystalline carotin. The observations were all made in a 10 cm. tube. Curves C and D are for 0.034 and 0.068 mgm. per cent solutions of carotin. Curves A and B are the liver pigment, which has been alleged to be carotin. The actual concentration of the liver pigment was of course, unknown.

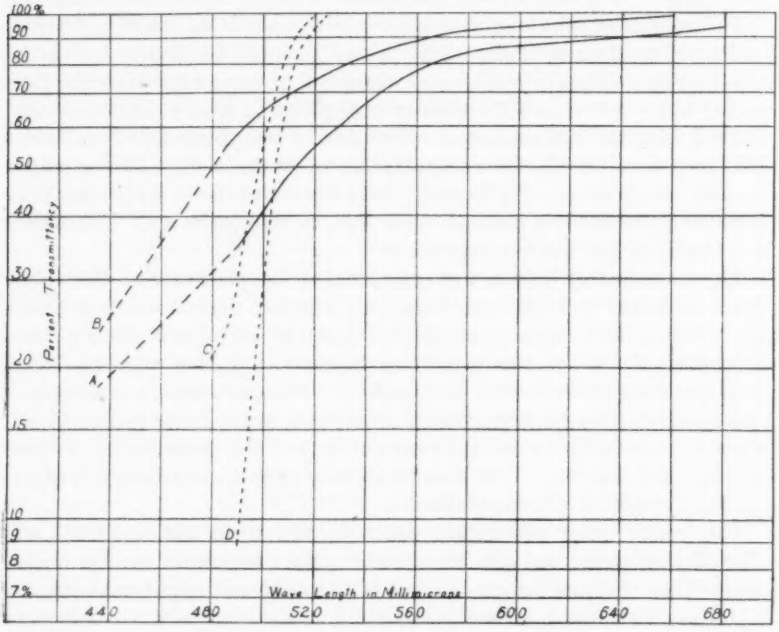

Fig. 1

solution B, however, was one-half the concentration of solution A, just as solution C for carotin was one-half the concentration of solution D. The beginning of curves A and B are broken lines because no observations were made between the point 435.8 mμ and 485 to 490 mμ. Above the latter wave lengths observations were made at intervals of 5 to 10 mμ.

The chart shows that the spectral transmittancy of the nonsaponifiable petroleum ether soluble pigment from pig's liver is totally dissimilar from

[4] This comparison was made for me by Dr. C. G. Ferrari, using a Keuffel and Esser spectrophotometer.

carotin in the visible region of the spectrum. This proves conclusively that the liver pigment is not carotin.

The liver pigment material was examined for other carotinoid-like properties. Its color is intensified in CS_2 but is not blood red like carotin. The pigment in this solvent is not adsorbed by $CaCO_3$ powder, thus resembling carotin. The pigmented residue gives a transient purple to maroon to brown color change with concentrated H_2SO_4. The pigmented material, like carotin, strongly reduces Fe_2Cl_6 crystals, but the pigment is not bleached as is carotin in this test. Concentrated HCl solution has no effect on an alcoholic solution containing the pigment. Carotin solutions slowly turn green in this test. The color of alcoholic solutions is very persistent, there being no visible bleaching after standing in the laboratory for several months. Carotin solutions bleach readily in a few weeks.

These results make it evident that some other explanation is required for the same effects of carotin crystals, carrots and pig's liver in the canine syndrome studied by Underhill and Mendel. The liver effects cannot be due to carotin. As Underhill and Mendel themselves suggest, but reject in their conclusions, their carotin crystals evidently carried some as yet unidentified substance or substances which were the effective agents.

CONCLUSION

There is present in pig's liver a minute quantity of non-saponifiable, petroleum-ether soluble pigment which resembles carotin in solubility and adsorption properties, which gives some color reactions similar to those given by the carotinoids, but which fails to give others. The pure pigment is much more stable to bleaching than carotin. The spectroscopic properties of the liver pigment are both qualitatively and quantitatively totally unlike carotin. The liver pigment, therefore, is not carotin. It cannot even be classed as a carotinoid because no known carotinoid fails to show spectroscopic absorption bands.

Acknowledgment. I gratefully acknowledge the kindness of Dr. C. G. Ferrari for the spectrophotometric measurements and the chart depicting them. The curves for carotin are published here for the first time. Should anyone have occasion to refer to them, credit should be given to Doctor Ferrari, not to the author.

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EFFECT ON RESPIRATION, BLOOD PRESSURE AND CAROTID PULSE OF VARIOUS INHALED AND INSUFFLATED VAPORS WHEN STIMULATING ONE CRANIAL NERVE AND VARIOUS COMBINATIONS OF CRANIAL NERVES

II. Vagus and Vagotomy Experiments

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Received for publication October 22, 1928

The purpose of this investigation is threefold: first, to determine the effect of inhaling strong and weak irritating vapors on respiration and circulation in a *vagal* animal, by which is meant an animal deprived of all effective sensory nerves supplying its upper respiratory passages (olfactory and trigeminal); second, to ascertain if there is any alteration of respiration and circulation during the inhalation of irritants in a *vagal* rabbit after double vagotomy; third, to repeat the experiments of several previous investigators on insufflation of irritating vapors into the trachea of vagotomized animals in which both positive and negative results have been obtained.

In attempting to solve the first problem suggested above, there is apparently no available technique (unless it be some complicated modification of the isolated head technique) for completely eliminating the possibility of humoral and secondary mechanical stimulation of the periphery or of humoral stimulation of the medulla centers. If negative results are obtained in the experiments of the second and third problems suggested, it may be inferred that humoral medulla-spinal stimulation is not a factor in the first problem. A previous paper and especially one to follow indicate that humoral and secondary mechanical factors take but little part in the alteration of respiration and circulation where these vapors were inhaled for relatively short periods of time.

The vagus changes in respiration following the blowing of an irritant into the trachea, as demonstrated by Zagari, consists of a considerable depression and acceleration. As reported by Roger, Mayer Magne and Plantefol, Craigie, Larsell and Burget, it includes a period of violent coughing or spasm followed by apnea or shallow respiration. Brodie and Russell's and Roger's blood pressure tracings show a distinct drop and a

strengthening of the pulse, and Craigie's tracings disclose a fall at the time of stimulation.

Effect of various inhalants on respiration, blood pressure and carotid pulse in vagal rabbits. Several days before these tests were made the olfactory tracts, the naso-ciliary and maxillary nerves were sectioned in the manner described in a previous report; severance of these nerves was previously found to be as effective in blocking trigeminal impulses from inhalations as division of the trigeminal roots intracranially. Thoracic respiratory and blood pressure tracings were taken as described previously with the animal under veronal-sodium hypnosis.

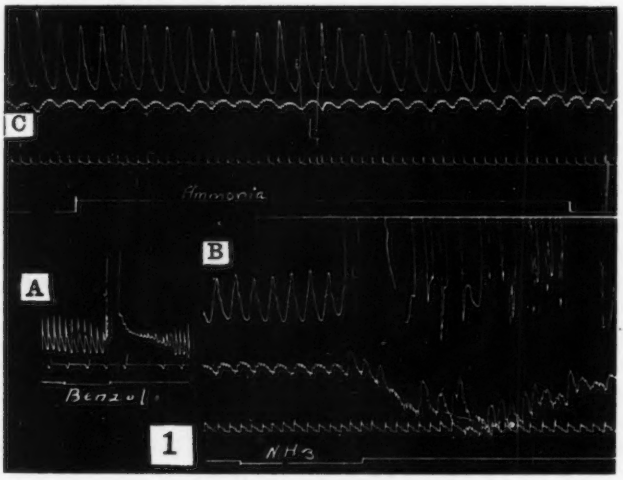

Fig. 1 A. *Vagal* rabbit, stimulation by inhalation, thoracic-respiratory graph, inspiration upstroke, time in 5 seconds; B and C, *vagal* rabbit, inhalation stimulation, thoracic-respiratory and blood pressure tracings, time in seconds; in C, depressors, vagi, and cervical sympathetics were cut.

In the first rabbit (563) the graphs were confined to respiratory tracings taken during the inhalation of ammonia, benzol and xylol from cones saturated with these substances. The ammonia cone was held about 7 cm. from the nostrils, the benzol about 4 cm., and the xylol about 3 cm. The benzol graph (fig. 1, A) reveals three distinct changes taking place in respiration. First, there is a short interval of depression. This gives way to a sudden expansion of the chest, starting during an expiration, it continues as a series of coughs or spasm. The extreme height of these forced movements of the lever was purposely stopped by a bar. This important phase is abruptly followed by a period of shallow and somewhat acceler-

ated excursions and a gradual return to normal. The graphs obtained during the inhalation of ammonia and xylol were somewhat similar. The chief difference is in the time of appearance of the altered respiration and the duration time of the coughing spell. The change appears within 9 seconds in the ammonia tracing, within 10 secones in the benzol record, and not until 20 seconds in the xylol graph. The coughing spell is longest in the ammonia record and shortest in the xylol. In the ammonia record there is some inhibition as well as depression in the first period, and only a moderate depression of the excursions following the coughing period.

In the second part of this experiment the depressors, vagi, and cervical sympathetics were sectioned at the level of the larynx, and respiratory tracings obtained during the inhalation of ammonia, benzol and acetic acid for periods of fifty seconds, as shown by the benzol graph (fig. 2, A), reveal respiration unchanged.

Both parts of this experiment were repeated in two other *vagal* rabbits with like results.

In *vagal* rabbits (571 and 572) this experiment was repeated and its scope was enlarged to include blood pressure tracings. The respiratory responses obtained during the inhalation of ammonia, aromatic spirits of ammonia, acetic acid, chloroform, oil of mustard, wintergreen, benzol and xylol are very similar to those obtained in the previous animals. Every record shows a considerable drop in blood pressure accompanied, usually, by some slowing and strengthening of the pulse.

In one graph (fig. 1, B) an ammonia cone was purposely held close to the nostrils to elicit a maximum stimulation. It is apparent that the reaction is concerned almost entirely with the interval of coughing, which appears in 9 seconds and lasts for 20 seconds. There is no depression or inhibition of the excursions preceding the period of coughing and only an acceleration of a few excursions immediately following. Meanwhile the blood pressure tracing discloses a drop of 20 mm. and there appears to be some slowing and strengthening of the pulse.

As in the previous animals the xylol reaction is the weakest and slowest in appearing.

After sectioning the depressors, vagi and cervical sympathetics, the same vapors inhaled for periods of 50 seconds evoke no change in respiration or pulse or any drop in blood pressure (see ammonia graph fig. 1, C). The apparent inhibition of respiration during the last part of this tracing is shown to be due to a speeding up of the drum. It is obvious from the blood pressure tracing (fig. 1, C) that there is no change for 32 seconds, the slight rise which occurs a little later may come from humoral stimulation of the medulla.

Effect from inhalation of weak and very weak irritants in vagal animals. It was stated in the previous experiment that xylol was one of the weakest

stimulants of the vagus, so that a weak xylol stimulation should yield a very weak vagal reaction, if it produces any at all. Figure 2, B was obtained while a cone, saturated with xylol, was held at some distance from the nostrils for 40 seconds. After 32 seconds of inhalation (allowance being made for the omitted portion of the record) there is a slight inhibition of respiration and a small drop in blood pressure without any change in the pulse. These changes are not thought to be of humoral medulla-spinal cord origin for the reason that none of the writer's graphs taken from vagotomized *vagal* rabbits show any alteration of respiration and blood pressure from inhalations of much stronger xylol vapor for a con-

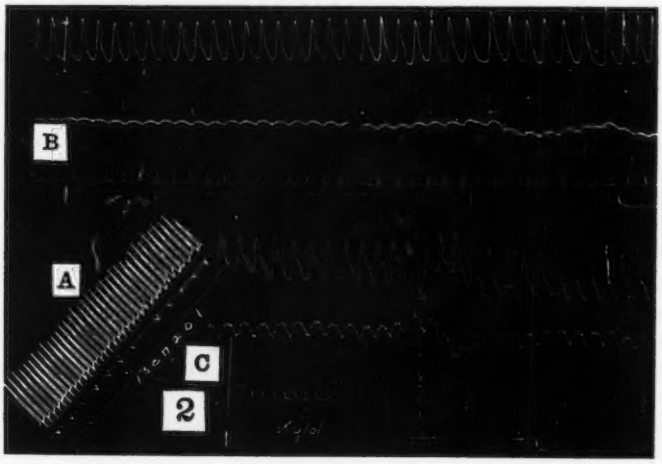

Fig. 2 A. Same as 1 A, except that depressors, vagi, and cervical sympathetics were severed; B and C, *vagal* rabbit, stimulations by low and very low concentrations of xylol inhaled, thoracic-respiration and blood pressure tracings (omitted portion in B represents 7 seconds), time in seconds.

siderably longer time. Also the drop in blood pressure speaks for a vagal stimulation.

A stronger xylol stimulus obtained from holding the cone closer to the nostrils (fig. 2, C) demonstrates the changes in respiration and blood pressure appearing within 16 seconds. The respiratory response, in the upper tracing, shows a cough reflex or spasm preceded and followed by short periods of depression. There is considerable drop in blood pressure and some slowing and strengthening of the pulse.

A typical vagal respiratory and circulatory response was obtained in a tracheotomized *vagal* rabbit with the laryngeal branch of the vagus intact by blowing large quantities of ammonia into both nostrils. Later when

the pharynx was slit above the larynx so that little or no ammonia reached the larynx, no vagus reaction was obtained from insufflation into the nostrils. This is apparently confirmatory of Kratschmer's results obtained by blowing an irritant directly into an isolated larynx.

Effect of insufflation of ammonia and other vapors into the trachea of vagotomized rabbits. Of the investigators who have stimulated the lower respiratory passages of vagotomized animals by blowing irritants into the trachea, Zagari, Mayer, Magne and Plantefol, and Larsell and Burget report no respiratory reaction; while Roger and Craigie obtained definite respiratory and blood pressure changes. Roger's figure 10 portrays considerable depression and inhibition in respiration and a drop in blood pressure during chloroform insufflation, and Craigie's graphs disclose the normal vagus changes slightly weakened. After a study of these publications it appeared likely that the difference in results might be largely attributed to variations in technique.

This experiment or problem passed through three different stages as unforeseen factors were encountered.

First,—a number of thoracic respiratory and blood pressure tracings were made from several vagotomized rabbits during the time that ammonia vapor was blown into the tracheal cannula through which the animal was breathing. These records demonstrate a varying amount of expansion of the thorax, a depression of respiration, and a fall in blood pressure. It was evident that these changes might be explained by local pulmonary changes offering a greater resistance to the respiratory movements of the thorax, due possibly to an increased expansion of the lungs from the insufflations or to a spasm of the constrictor muscles of the trachea and bronchi. With these possibilities in mind it became necessary to pay more attention to air controls and to the amount of pressure used during insufflation.

Second,—the previous procedure of taking thoracic and blood pressure records during insufflations into the trachea of rabbits deprived of their depressors, vagi, and cervical sympathetics was repeated and expanded to include a respiratory tracing taken from the trachea. The apparatus for obtaining the tracheal record consisted of a Y-shaped tracheal cannula, one external arm of which was connected to a previously described blowing bottle by a rubber tube with a pinch clamp, and the other external arm was in direct communication with a large air bottle and recording tambour. The bottle in addition to supplying some air for ventilation served as a sort of buffer. The wounds in the neck, the mouth, and the nostrils were covered with cotton.

The procedure of insufflation was as follows: the animal breathed from the air chamber for a short interval, then air and insufflated vapor for periods of from 3 to 17 seconds, and finally from the air chamber. In a

few tests this technique was varied by the attachment of two blowing bottles to one arm of the cannula, so that air blown from the first bottle into the trachea could be followed immediately by an irritating vapor from the second. A large number of simultaneous thoracic-respiratory, tracheal-respiratory, and blood pressure tracings were obtained from five rabbits during the insufflation of ammonia, acetic acid, ether, and especially air, under great variations of pressure.

These records reveal that there is a direct relationship between any given tracheal respiratory excursion and the corresponding thoracic ex-

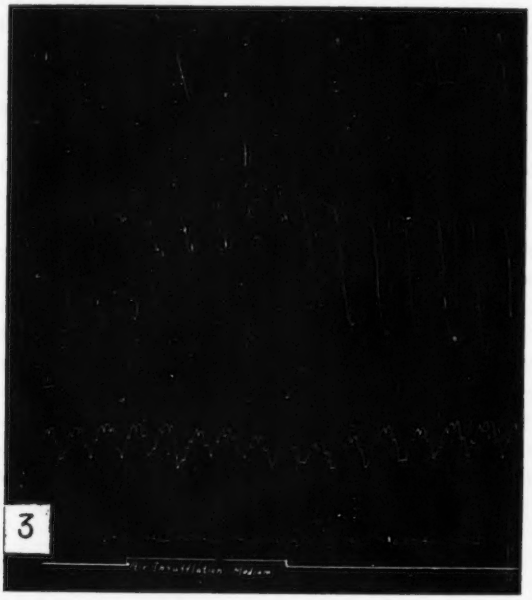

Fig. 3. Vagotomized rabbit, tracheal insufflation, thoracic-respiratory, tracheal-respiratory, and blood pressure tracings, time in seconds.

cursion, and it is immaterial whether this record is from insufflation of an irritant or air. An air control graph (fig. 3) demonstrates that a slight increase in air pressure is followed by some increase in the depth of the first thoracic respiratory excursion. After a certain point has been reached in the expansion of the lungs and thorax, a gradual increase in pressure elicits a gradual expansion of the thorax and a gradual reduction in the depth of thoracic respiration. In some records this has been increased to the extent of bringing pulmonary and thoracic respiration to a standstill. By varying the pressure in an air control graph, it is possible to duplicate any graph obtained from any irritant.

With the technique employed there is some indication of greater expansion in the lungs by some vapors (ether) than there is with some others under the same pressure.

Practically all of these records show some drop in blood pressure and no pulse change. In general, there is a considerably greater drop in the graphs obtained during the insufflation of an irritant than there is for air. The contour of the blood pressure curve appears to conform very closely to the curve of the general expansion of the thorax as is shown in figure 3.

Third,—in two rabbits the above stimulation technique was considerably modified. In place of insufflating the vapors into the tracheal cannula, the bulb was removed from the blowing bottle and the animal was permitted to inhale through the cannula air saturated with ammonia, acetic acid, and ether for periods of from 12 to 18 seconds. After each inhalant, air was inhaled through water for the same length of time. There can be no question but that this mode of procedure flooded the lungs with large quantities of air well saturated with these vapors. A comparison of any irritant graph with its air control discloses no difference. Both respiratory tracings of all the records show some expansion during the time of inhalation. Ordinarily blood pressure shows no changes in either record, but sometimes there is a slight drop in both tracings.

Comment. Two deductions appear to be fairly well warranted by the results obtained from the foregoing experiments—a, that certain nerves, supposed by Barry, Pike and Coombs, and Möllgaard to carry impulses from the lungs to spinal cord by way of the upper thoracic and lower cervical spinal nerves, are not stimulated to produce respiratory changes by large quantities of air containing a high concentration of very irritating vapors; b, that relatively short inhalations or insufflations of very irritating vapors do not cause a humoral medulla-spinal stimulation affecting respiration.

SUMMARY AND CONCLUSIONS

Moderately strong inhalations of various irritating vapors by way of the nose in the so-called *vagal* rabbits, animals having no protection from the olfactory or trigeminal nerves, produce somewhat similar respiratory and vascular changes to those described by others from blowing these irritants into the trachea.

This alteration of thoracic respiration consists of 1, a short period of depression or depression and inhibition; 2, a spasm or period of coughing, depending in length and strength on the irritant used; 3, a period of more or less depression with a gradual return to normal. The important character is the period of coughing.

Blood pressure drops in proportion to the strength of the irritant used and to the general expansion of the thorax. There is generally some slowing and strengthening of the carotid pulse.

A very powerful thoracic respiratory reaction from inhalations in a *vagal* rabbit consists of the immediate appearance of the coughing spell; while a very weak reaction shows only a slight inhibition of respiration, a little drop in blood pressure and no pulse changes.

Changes in respiration and circulation are elicited from inhalations of ammonia, acetic acid, formalin, oil of mustard, wintergreen, chloroform, ether, benzol and xylol.

After sectioning the depressors, vagi, and cervical sympathetics at the level of the larynx, any of the above vapors could be inhaled through the nostrils by a *vagal* rabbit for fifty seconds without any alteration of respiration or drop in blood pressure.

Insufflation of irritating vapors into the trachea of vagotomized rabbits is followed by a response in respiration and circulation very similar to that described by Roger for chloroform, consisting of a general expansion of the thorax, a depression of respiration, and a drop in blood pressure. Evidence is produced which shows that these respiratory changes in the writer's animals are probably caused by an increase in the intrapulmonary pressure resulting from the insufflations.

Vagotomized rabbits, inhaling through the trachea large quantities of air containing a high concentration of irritating vapors, show no greater respiratory and circulatory changes than when inhaling unmixed air under the same conditions.

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THE GLYCOGENIC FUNCTION OF SKELETAL MUSCLE IN THE DEHEPATIZED DOG, WITH SPECIAL REFERENCE TO THE RÔLE OF INSULIN THEREIN

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Received for publication October 13, 1928

Problems in carbohydrate metabolism have usually been investigated by studying changes in the blood sugar, in the liver and muscle glycogen, and in the respiratory quotient. The pioneer work on insulin confined itself to these phases of study, and surprising as it may seem, practically all we know about carbohydrate metabolism has been derived by correlation of these three variables. More recently use has been made of changes taking place in the inorganic phosphate and the lactic acid metabolism of the organism. On the whole, therefore, one's stock-in-trade for investigating a given problem in carbohydrate metabolism is remarkably small, and in investigating the mode of action of such a substance as insulin, the researches have of necessity confined themselves to such problems as whether it raises the respiratory quotient, and whether it leads to a deposit of muscle and liver glycogen. It is not obvious, however, how any of these methods can solve the problem of the fundamental action of insulin since it has not been proved that the difference between diabetic and non-diabetic skeletal muscle can be explained in terms of any of these variables. In other words, it is not yet known what constitutes the fundamental difference between the diabetic and the non-diabetic state.

We have been concerned for several years with the glycogenic function of the skeletal muscle and, accordingly, were much interested in the publication of Best, Dale, Hoet and Marks, whose work in this field must be considered a distinct contribution to the physiologic action of insulin. They showed that the sugar which disappears under the action of insulin in eviscerated cats can be accounted for completely on the basis of oxidation or deposition as muscle glycogen. Best, Hoet, and Marks had previously shown that unless insulin is injected into the preparation, the infusion of dextrose is not followed by the appearance of more muscle glycogen. The latter observation has been repeated by Choi who also showed that the same was true of depancreatized decapitated cats. Best, Dale, Hoet and Marks, on the basis of their experiments, suggested that

the action of insulin is twofold: 1, it inhibits hepatic glyconeogenesis from protein and fat, a view especially championed by Laufberger, and 2, it stimulates the synthesis of muscle glycogen. The meaning is not clear with regard to what they consider the function of insulin in promoting the deposit of muscle glycogen. They believe that the action of large doses of insulin is manifested by an exaggeration of its normal action and presumably, therefore, in the normal animal they consider insulin to be essential for the deposition of muscle glycogen. Two criticisms, perhaps minor ones, may be made of these splendidly conceived experiments: 1, the presence of the liver in the peritoneal cavity is undesirable because the percentage of hepatic tissue still functioning is not known; this criticism acquires greater significance in view of our work showing that the liver is perhaps intimately concerned with the action of insulin, either directly or indirectly; and 2, the glycogenic function of skeletal muscle in the flaccid spinal preparation may not be comparable to that of an intact animal. For experiments of this nature it would seem desirable to start with animals whose muscle glycogen had been reduced in amount. If a muscle which is initially deficient in glycogen fails to make this substance in the presence of a plethora of glucose, the inference is strong that some factor is lacking for this transformation, which inference need not necessarily follow in muscles with a normal glycogen content, since this is probably well above that required by denervated muscle. Somewhat vitiating this criticism, however, is the familiar observation that denervated muscle is richer in glycogen than is functionally active muscle.

The conclusion that glycogen, in order to be deposited, requires insulin is a very puzzling one since on discontinuing the administration of food and insulin to depancreatized dogs, the glycogen content of the muscles is not diminished. Thus five days after the last administration of food and insulin Macleod and Markowitz found the gastrocnemius muscle to contain 0.73 per cent glycogen, the rectus femoris 0.62 per cent, the left ventricle 1.40 per cent, and the liver practically none (Chaikoff, 1927). The well-known increase of glycogen in heart muscle following pancreatectomy is difficult to explain on the basis that insulin is necessary for the formation of muscle glycogen (Cruickshank, 1913).

Barbour, Chaikoff, Macleod and Orr, using the standard white rat, found that when moderate amounts of insulin were injected one hour after the feeding of a standard meal, the glycogen in the liver was definitely diminished at all periods up to three hours after the injection of insulin. The glycogen in the muscles meanwhile was unchanged. These careful observations cannot easily be interpreted on the basis that in the intact animal insulin normally brings about a deposit of muscle glycogen.

With the foregoing considerations in mind we undertook experiments similar to those of Best, Hoet and Marks, making use of the dehepatized

dog and taking the precaution of attempting to lower the amount of muscle glycogen by various procedures.

METHODS OF EXPERIMENTATION. The technic for hepatectomy has been described in previous papers from this laboratory. In several experiments the animal was dehepatized by the method of Markowitz and Soskin with satisfactory results.

The size of the dog as compared with the cat, and also the fact that the animal was allowed to recover from the anesthetic precluded the use of a whole muscle for glycogen analysis, and thus it was necessary to perform a control experiment, comparing the glycogen content of samples of corresponding muscles from opposite sides of the body (expts. 10 and 11). It might be stated that the results thus obtained agree somewhat better than those of Cramer who, however, worked with the older Brücke method. The agreement is not quite so good as that obtained when the whole muscles of the opposite sides of the body of a cat were used, as reported by Best, Hoet and Marks.

The first samples of muscle were taken with aseptic precautions under ether anesthesia through an incision over the medial aspect of the thigh. The muscle was gently freed from the overlying fascia and when all was ready as much of it as was deemed feasible was rapidly excised. In the case of the sartorius, and also the semimembranosus, the amount excised was sometimes about 10 grams but usually it was 6 to 8 grams, depending on the size of the dog. In the case of the other muscles the quantity obtained was usually more than 10 grams. The muscle so obtained was rapidly stripped of fat and fascia, divided into about two equal parts for duplicate estimations, quickly weighed on a torsion balance, and dropped into hot caustic potash. This procedure was carried out in the operating room after the completion of the major operation and while the animal was still under the anesthetic. Each specimen was placed in the caustic potash within about a minute, at most within two minutes, by which time the next muscle was ready for excision. The sartorius, vastus medialis, semimembranosus, and biceps femoris were the muscles usually employed and the same muscles were removed under local anesthesia at the end of the experiment. The glycogen was estimated by Pflüger's technic, the sugar being estimated by the Shaffer-Hartmann method, using Duggan and Scott's tables. The analytic portion of this technic was checked by the quantitative recovery of added glycogen. Although the technic employed by us permitted of some glycogenolysis, this factor was strictly comparable in all our experiments, as is shown by the reasonable agreement of the figures in the control experiments mentioned. It should be borne in mind that although corresponding whole muscles from opposite sides of the body may have nearly the same glycogen content, this need not be true of pieces removed under the conditions of our experiments.

In many of these experiments the attempt was made to render the muscles of the animal deficient in glycogen by fasting, shivering, and muscular exercise. In this we were not altogether successful. In the case of the dog there is evidently an efficient mechanism for replacing glycogen as fast as it is used. Older observations to the effect that dogs can be made glycogen-free by this procedure are possibly not correct, due regard not having been made for the extreme rapidity of post-mortem glycogenolysis in injured muscle tissue.

The problem was studied under the following headings: 1, the influence of injecting a dehepatized dog with large quantities of glucose, confirming the observations of Bollman, Mann and Magath (expts. 1 and 2); 2, the influence of injecting large amounts of glucose without insulin into a dehepatized depancreatized dog (expt. 3); 3, the influence of injecting large amounts of glucose and insulin into a dehepatized depancreatized dog (expts. 4 and 5); 4, the influence of injecting large amounts of glucose into a depancreatized dog whose kidneys had been excised to prevent the loss of sugar in the urine (expts. 6, 7 and 8); 5, the muscle glycogen content of a fed dog several days after pancreatectomy, the dog having been strenuously treated before operation to deplete his glycogen stores (expt. 9), and 6, various control experiments (expts. 10, 11 and 12).

COMMENT. It is perhaps necessary to point out that these animals appeared normal after the operation and were physiologic preparations in most senses of the term.

The results clearly show, in confirmation of those of Bollman, Mann and Magath, that the giving of a sufficient amount of glucose to a dehepatized dog is followed by the formation of a large quantity of muscle glycogen. To demonstrate this it is essential that the animal should be given more glucose than its caloric requirement, which suggests that possibly a dehepatized dog can obtain its energy only from the oxidation of carbohydrate. The older work by Laves, and by Kausch on the glycogenic function of muscle in dehepatized hens and geese led these workers to conclude that in the absence of the liver the giving of glucose is ineffectual in causing muscle glycogen to be deposited. Our experiments supply an explanation for the results of Laves' and of Kausch's very able experiments since analysis of their protocols shows that they either injected too little glucose or else gave it by stomach tube under conditions in which not enough was absorbed during the life of the animal. Moreover, dehepatized hens and geese do not tolerate a sufficiently large quantity of glucose to act as test objects for such a problem.

The foregoing experiments are in distinct contrast to those in which the pancreas and liver were simultaneously excised, the kidneys being also removed to prevent loss of sugar in the urine. The giving of glucose in any dose was not followed by storage of muscle glycogen unless relatively

enormous doses of insulin were also injected. In the case of a dog weighing 14.1 kgm. (expt. 4), even when more than 400 units of insulin was injected with the glucose, there was a striking fall in muscle glycogen. An experiment, in which a partially dehepatized dog (expt. 12) was given similar quantities of glucose, showed a striking increase in muscle glycogen. We realize, however, that this is an imperfect control. There remains the observation, therefore, that when enormous quantities of insulin are given to a dehepatized depancreatized dog, together with enough glucose, muscle glycogen is laid down. The experiments demonstrate definitely that although insulin does functionate in a liverless dog, its function is by no means so marked as in a diabetic but otherwise intact dog that following the injection of 12 units of insulin, can take care of a meal of 200 grams meat, 50 grams raw pancreas and 50 grams cane sugar, with practically no glycosuria. This point is one that cannot be ignored in attempting to localize the action of the insulin. According to Best, Dale, Hoet and Marks, insulin acts in the normal organism by inhibiting hepatic glyconeogenesis from protein and fat, and by bringing about a deposit of muscle glycogen. On this hypothesis, 12 units of insulin should have as marked an effect on a dehepatized depancreatized dog as on a diabetic but otherwise normal dog. Our results, which showed that such is not the case, are inexplicable on the basis of their hypothesis.

The relatively resistant state of the dehepatized dog to insulin suggests strongly either that insulin has its major action in the liver or that the liver secretes a compound into the blood stream that is essential for the action of insulin in the tissues. The resistance of the dehepatized dog to insulin is well illustrated by the following experiment:

Following hepatectomy a dog weighing 1.5 kilos was given 0.25 gram of glucose for each kilogram of body weight each hour, for several hours, and the rate of fall of the blood sugar was noted by frequent estimations of the blood sugar. One hundred units of insulin was then injected with the same dose of glucose. The blood sugar instead of falling more rapidly showed a retarded rate of fall. In fact, in two experiments we were impelled to test the insulin on a normal dog to assure ourselves of its potency.[1] It is necessary to point out, however, that this is an exceptional response; there being in most instances a definite effect from insulin in the dehepatized dog when 5 units for each kilogram of body weight is injected. Whether or not such a dose of insulin will influence the blood sugar of a dehepatized dog seems to depend on the amount of glucose previously injected into the animal and, perhaps for the same reason, on the length of time that has elapsed since hepatectomy. Dogs that have received much glucose, or dogs that are injected with insulin six or eight hours after

[1] Fresh insulin from the Lilly laboratories was always used.

hepatectomy may not give any detectable response in the blood sugar even following much larger doses of insulin than 5 units for each kilogram of body weight. We are, of course, not justified in being dogmatic, but it seems clear to us that the action of insulin on carbohydrate in the intact animal is dependent on a third factor. The results of Jordon and of Allan on the glucose equivalent of insulin, in terms of grams of glucose metabolized, may be readily interpreted on this basis. Working with depancreatized dogs on a constant diet, Allan found that the quality of glucose metabolized for each unit of insulin injected varied inversely with the amount of insulin injected. Similarly, working with a Woodyatt perfusion pump on normal dogs, Jordon found that when the rate of injection of glucose was high (3.6 grams for each kilogram of body weight for each hour), the giving of 100 units of insulin was without effect on the glycosuria. The results of these workers may be considered as evidence of the existence of an unknown factor which is necessary for the action of insulin on carbohydrate.

The results obtained with depancreatized nephrectomized animals fal in line with our foregoing experiments. The giving of glucose immediately after operation is occasionally followed by a definite increase in muscle glycogen, but when several hours are first allowed to elapse the giving of glucose is not followed by this result.

In cases in which dogs deficient in glycogen were depancreatized and fed for several days after operation, there were often definite increases in muscle glycogen. About half the experiments of this kind were negative in that some muscles at the end of the experiment showed an increase and some showed about the same quantity of muscle glycogen.

On the whole we may sum up the results fairly by stating that although muscle glycogen can readily be deposited when a dehepatized dog is given at least 1 gram of glucose for each kilogram of body weight each hour, the presence of the pancreas is necessary for this effect, for in the absence of the pancreas the giving of glucose to a dehepatized dog is not followed by a deposit of muscle glycogen unless much insulin is also injected. These observations are in general supported by the other experiments of the series in that it was difficult to demonstrate the deposit of muscle glycogen in the absence of the pancreas.

Our experiments can be criticized from two standpoints: 1, the introduction of so much glucose in such a short time causes a very considerable upset in the water balance of the organism, especially in the depancreatized preparations in which the hyperglycemia was very great and the blood often distinctly watery; the failure of such preparations to lay down muscle glycogen may be dependent not on the absence of insulin per se, but rather on the unphysiologic conditions of the animal, and 2, the muscle, teleologically speaking, may not need the extra glycogen so that even in the

presence of surplus of sugar, more is not stored; this criticism presupposes that insulin either increases the glycogen requirements of the muscle or else brings the glucose into more "efficient" contact with enzymes that transform it into glycogen.

In the face of these criticisms caution should be used in applying our results to what obtains in an intact animal. That the extrahepatic function of insulin is concerned purely with glycogen formation is difficult to rationalize with the following: 1, it fails to explain the changes in blood phosphate accompanying the action of insulin unless it is assumed that the phosphate accompanies the glycogen, in which case one encounters the difficulty of explaining how so much phosphate can disappear when glycogen presumably is accompanied by only traces, and 2, it fails to explain why the heart-lung preparation from a diabetic dog does not survive very long and why insulin should revive it, when presumably such a heart has more than a normal supply of glycogen. To put the matter otherwise: it is difficult to believe that the presence or absence of the diabetic state in a muscle is entirely a function of its glycogen content.

It would be idle to speculate further on the significance of our observations and those of Best, Dale, Hoet and Marks in the present limited state of our knowledge of carbohydrate metabolism. We are not inclined to emphasize these observations in attempting to solve the antidiabetic action of insulin, since the general action of insulin in a normal dog is not the same as in a dehepatized dog.

The relative refractoriness of the dehepatized dog to insulin may be compared with the similar behavior of the diabetic patient under certain conditions: 1, when a severe infection such as pneumonia develops in a diabetic patient the insulin requirement may reach colossal proportions; 2, very rarely cases of genuine diabetes mellitus are reported in which little benefit is derived from insulin, the blood sugar failing to respond even to massive doses, and 3, Hepburn, Latchford, McCormick and Macleod showed that during ether anesthesia the injection of as much as 100 units of insulin into a diabetic dog does not result in a fall of the blood sugar; this has its analogy clinically, for when acidosis develops in a diabetic patient following an operation he is less amenable to insulin treatment than he would have been had the treatment been instituted before operation.

SUMMARY

When glucose is given to a dehepatized dog in quantities of at least 1 gram for each kilogram of body weight for each hour there is a definite rise in muscle glycogen (confirming observations by Bollman, Mann and Magath). This rise in muscle glycogen cannot be demonstrated when the pancreas is excised together with the liver. If, however, large quantities of insulin are also given there may be a definite rise in muscle glycogen in

the depancreatized dehepatized animal. In one case, in spite of massive dosage with insulin, the giving of glucose did not result in a deposit of muscle glycogen. The amount of insulin to be effective in this regard was very large under certain conditions. When insulin was injected into dehepatized dogs several hours after hepatectomy and after several administrations of glucose, it could often not be demonstrated that it had any hypoglycemic action unless relatively enormous doses of insulin were injected. It is suggested, on the basis of these data, that perhaps the liver plays a major part in the action of insulin in the intact animal. The suggestion is also made that the action of insulin on carbohydrate in the intact animal is possibly dependent on a third, and as yet unknown, factor.

It was found difficult to demonstrate glycogen formation when depancreatized nephrectomized animals were injected with large quantities of glucose. Some undoubted cases of glycogen formation are reported.

The experiments may be taken as decisively confirming the observations of Best, Dale, Hoet and Marks, although one is inclined to question their interpretation on the grounds that the action of insulin in a liverless preparation is quantitatively different from its action in normal animals.

PROTOCOLS. *Experiment 1.* January 17, 1927, a reverse Eck fistula was satisfactorily made, with the usual technic, on a male shepherd dog weighing 13 kgm. July 19 the animal was in excellent condition and the abdomen was opened and the portal vein ligated. December 1 the animal was in excellent condition. A week previous to this date food was withdrawn and the animal permitted to be in the cold for several hours a day. November 26, 27, 28 and 29, 500 cc. of milk was given. The animal was fasted all day on November 30, and the final operation was performed on the morning of December 1. The liver and both kidneys were removed and the usual specimens of muscle taken. The liver was rather small for a dog of this size (weight 200 grams). The animal was etherized at 8:40 a.m., the incision was made at 8:45 a.m., and the liver was out at 8:55 a.m. The ether was off at 9:20 a.m., and the last stitch was taken at 9:25 a.m. The animal weighed 10.6 kgm. before operation and 10 kgm. after operation.

At 8:30 a.m. the blood sugar was 0.088 per cent. At 9:15 a.m. the animal began to twitch while still on the table; accordingly it was given 1 gram of glucose intravenously for each kilogram of body weight. At 9:45 a.m. the injection of glucose was repeated. At 10:15 a.m., 10:45 a.m., 11:15 a.m., 11:45 a.m., 12:15 p.m., 12:45 p.m., 1:15 p.m., 1:45 p.m., 2:15 p.m., 2:45 p.m., 3:15 p.m., 3:45 p.m., 4:15 p.m., 4:45 p.m., 5:15 p.m., 5:45 p.m., 6:15 p.m., 6:45 p.m., 7:15 p.m. and 7:45 p.m. the animal was given 1 gram of glucose intravenously for each kilogram of body weight in a 50 per cent solution. The blood sugar at 2:44 p.m. was 0.676 per cent at 4:15 p.m. 0.724 per cent and at 9:10 p.m. 1.12 per cent. The animal was in excellent condition throughout the experiment. At 9:20 p.m., under local anesthesia, samples of muscle were taken from the fore and hind limbs for glycogen estimation, after which the animal was killed. Necropsy was negative (table 1).

Comment. The experiment showed conclusively that large quantities of muscle glycogen can be made in the absence of the liver.

Experiment 2. October 13, 1926, a reverse Eck fistula was made with the usual technic on a male black and white bull terrier, weighing 15 kgm. October 6 the dog weighed 19.2 kgm. The abdomen was opened and the portal vein ligated. November 21, 1927, the animal had been in excellent condition since the last operation. On the evening of November 17 it was placed on a fast and food was not given before operation, although water was not withdrawn. For about four to five hours each on the succeeding days, November 18, 19 and 20, the animal was placed outside.

TABLE 1

Experiment 1

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.590	0.732
	0.602	0.700
Rectus femoris	0.308	1.342
	0.278	1.320
Vastus internus	0.256	1.068
	0.276	1.138
Left biceps femoris		1.440
		1.242
Right biceps femoris		1.070
Right pectoralis major		0.689
		0.579

TABLE 2

Experiment 2

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.367	
	0.365	
Rectus femoris	0.250	0.262
	0.235	
Vastus internus	0.253	0.192
	0.277	
Left sartorius*	0.181	
	0.156	
Pectoralis major*	0.153	0.362
	0.168	0.334
Left biceps femoris		0.852
		0.805
Right biceps femoris		0.768
		0.906

* These muscles were removed at 10:40 a.m. before the animal had received any glucose.

The weather, however, was not very cold. November 20, after a blood specimen had been taken, the animal was etherized and the liver removed with the usual technic. Both kidneys were also removed. Operation was difficult although only a small amount of blood was lost. Specimens from the usual three muscles of the right thigh were secured. Ether was given at 8:35 a.m., and the incision made at 8:40 a.m.; the liver was out at 8:55 a.m. The ether was off at 9:25 a.m., and the last stitch taken at 9:25 a.m. The animal weighed 17.7 kgm. before operation and 17.0 kgm. after operation. The liver weighed 290 grams.

At 8:30 a.m. the blood sugar was 0.066 per cent. At 10:40 a.m. the animal, which

had previously been in good condition, suddenly began to twitch all over and rapidly became semiconscious. The blood sugar at this time was 0.039 per cent. Specimens of muscle were removed under procaine anesthesia for glycogen estimation and at 10:50 a.m. it was injected with 0.5 gram of glucose for each kilogram of body weight. The animal recovered immediately. At 11:20 a.m. 0.5 gram of glucose for each kilogram of body weight in 25 per cent solution was injected. At 11:55 a.m., 12:25 p.m., 12:55 p.m., 1:25 p.m., 1:55 p.m., 2:25 p.m., 2:55 p.m., 3:25 p.m., 3:55 p.m., 4:25 p.m. and 4:55 p.m. injections were repeated. At 4:54 p.m. the blood sugar was 0.250 per cent. At 5:25 p.m. the injection was repeated. At 6:25 p.m. and 6:55 p.m. the injection was repeated. At 6:45 p.m. the blood sugar was 0.236 per cent. At 7:25 p.m. the injection was repeated. At 8:00 p.m. the blood sugar was 0.200 per cent. Specimens of muscle were taken under procaine anesthesia and the animal was killed (table 2).

Comment. This animal had been fasted and placed in the cold for a number of days in order to bring down the glycogen level in the muscles previous to hepatectomy. In this we were more successful than usual, although very definite quantities of glycogen were present. The experiment demonstrates that a dehepatized dog can make muscle glycogen when given enough glucose. Necropsy showed nothing that would vitiate this experiment.

Experiment 3. October 20, 1926, a reverse Eck fistula was performed with the usual technic on a male bull terrier, weighing 16.5 kgm. October 6, 1927, the portal vein was ligated. November 1, the pancreas, liver and the usual specimens of muscle from the right thigh were removed. The weight of the pancreas was 30 grams. The weight of the liver was 385 grams. Ether was given at 8:40 a.m.; incision was made at 8:50 a.m., and the liver was out at 9:10 a.m.; the ether was off at 9:15 a.m., and the last stitch was taken at 9:20 a.m. The animal weighed 14.9 kgm. before operation, and 14.0 kgm. after operation.

At 8:30 a.m. the blood sugar was 0.066 per cent and at 9:25 a.m. it was 0.084 per cent. The animal was given 0.13 gram of glucose intravenously for each kilogram of body weight in a 25 per cent solution. At 9:55 a.m. the blood sugar was 0.066 per cent. The injection of glucose was repeated. At 10:25 a.m. the blood sugar was 0.08 per cent. The injection was repeated. At 10:55 a.m. the blood sugar was 0.08 per cent. The injection was repeated. At 11:00 a.m. 0.25 gram of glucose for each kilogram of body weight was injected. At 11:30 a.m. the blood sugar was 0.190 per cent. The injection was repeated. At 12:00 m. the injection was repeated. At 12:10 p.m. under procaine anesthesia a large piece of the biceps femoris was removed for glycogen estimation. A third duplicate was done to which was added 5.81 mgm. of glycogen. On the assumption that the concentration of glycogen in this third duplicate was the average of the other two, 5.67 mgm. of glycogen were recovered. At 12:30 p.m. 0.5 gram of glucose for each kilogram of body weight was injected. The blood sugar was 0.264 per cent. At 1:00 p.m. it was 0.266 per cent; the injection was repeated. At 1:30 p.m. the blood sugar was 0.400 per cent; the injection was repeated. At 2:00 p.m., the blood sugar was 0.336 per cent; the injection was repeated. At 2:30 p.m. the blood sugar was 0.528 per cent; the injection was repeated. At 3:00 p.m. the blood sugar was 0.524 per cent; the injection was repeated. At 3:20 p.m. the blood sugar was 0.768 per cent; the injection was repeated. At 4:00 p.m. the blood sugar was 0.768 per cent; the injection was repeated. At 4:30 p.m. the blood sugar was 0.784 per cent; the injection was repeated. At 5:00 p.m.

TABLE 3

Experiment 3

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.859	0.116
	0.902	0.105
Rectus femoris	0.632	0.068
	0.615	0.068
Vastus internus	0.452	0.066
	0.412	0.067
Biceps femoris*	0.542	0.166
	0.488	0.341

* Removed at 12:10 p.m.

TABLE 4

Experiment 4

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.828	0.360
	0.804	0.320
Rectus femoris	0.612	0.159
	0.565	0.138
Vastus internus	0.550	0.158
	0.688	0.150
Biceps femoris*	0.938	0.798
	0.950	0.798

* Removed at 1:50 p.m.

TABLE 5

Experiment 5

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.224	0.610
		0.567
Rectus femoris	0.155	0.477
	0.167	0.642
Vastus internus	0.164	0.574
		0.721
Gracilis	0.214	0.065

TABLE 6

Experiment 6

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.337	0.351
	0.344	
Rectus femoris	0.257	0.412
	0.257	0.404
Vastus internus	0.259	0.240
		0.258
Liver	0.205	0.890
	0.200	0.864
Biceps femoris*	0.308	0.300
	0.330	0.330

* Removed at 1:45 p.m.

the injection was repeated. At 5:30 p.m. the blood sugar was 0.792 per cent. At 6:00 p.m. the injection was repeated. At 6:30 p.m. the blood sugar was 0.800 per cent; the injection was repeated. At 7:00 p.m. the blood sugar was 0.888 per cent. At 7:05 p.m. samples of the muscle were removed under local anesthesia, the animal being in good condition at this time. A few minutes later the animal died suddenly (table 3). At necropsy nothing was found that would vitiate this experiment.

Comment. In spite of the administration of 1 gram of glucose for each kilogram of body weight each hour the muscle glycogen fell decisively.

Experiment 4. October 12, 1926, a reverse Eck fistula was made with the usual technic on a female mongrel airedale, weighing 12.5 kgm. October 4, 1927, the animal had remained in excellent condition; it weighed 16.3 kgm. The abdomen was opened and the portal vein ligated. The color of the intestine did not remain satisfactory, and the spleen was also removed. November 3, the weight was 15 kgm. The animal had remained in good condition since the last operation. A specimen of blood was taken and under ether anesthesia the pancreas and liver were removed. It was necessary to put a loose ligature around the pedicle of the right kidney to prevent hemorrhage from the thin-walled capsular veins. Ether was given at 8:40 a.m.; the incision was made at 8:50 a.m.; ether was off at 9:20 a.m.; the last stitch was taken at 9:25 a.m. and the liver was out at 9:10 a.m. The animal weighed 14.1 kgm. after the operation. The liver weighed 320 grams, and the pancreas weighed 35 grams.

At 8:30 a.m. the blood sugar was 0.080 per cent. At 9:25 a.m. the animal was given 30 cc. of 25 per cent glucose and 6 units of insulin intravenously. At 10:00 a.m. the blood sugar was 0.160 per cent; the injection was repeated. At 10:30 a.m. the blood sugar was 0.260 per cent; the injection was repeated. At 11:00 a.m. the blood sugar was 0.266 per cent; the injection was repeated. At 11:30 a.m. the blood sugar was 0.384; the injection was repeated and 8 units of insulin given. At 12:00 m. the blood sugar was 0.400 per cent; the injection was repeated. At 12:30 p.m. the blood sugar was 0.444 per cent; the injection was repeated and 10 units of insulin given. At 1:00 p.m. the injection of glucose was repeated and 14 units of insulin given. At 1:30 p.m. the blood sugar was 0.498 per cent. The injection was repeated and 10 units of insulin given. At 1:50 p.m., under local anesthesia, a piece of biceps femoris was excised for glycogen estimation. At 2:00 p.m. the injection of glucose was repeated and 10 units of insulin given. At 2:30 p.m. the injection of glucose was repeated and 20 units of insulin given. At 3:00 p.m. the blood sugar was 0.500 per cent; the injection of glucose was repeated and 14 units of insulin given. At 3:40 p.m. the injection of glucose was repeated and 114 units of insulin given. At 3:50 p.m. the animal urinated; the urine gave a brick red reduction with Benedict's solution. At 4:00 p.m. the injection of glucose and 14 units of insulin was repeated. At 4:30 p.m. the injection of glucose and insulin was repeated. At 5:00 p.m. the blood sugar by the Shaffer-Hartmann method gave a scarlet red reduction without a trace of blue in the reagent. The blood sugar was probably more than 0.5 per cent. At 5:30 p.m. the injection of glucose and insulin was repeated. At 5:40 p.m. 100 units of insulin was injected intravenously. At 6:00 p.m. the injection of glucose and 14 units of insulin was repeated. At 6:45 p.m. the blood sugar was as in the last sample. The injection of glucose and insulin was repeated. At 6:50 p.m. specimens from the four muscles were removed for glycogen estimation under local anesthesia (table 4). At 7:00 p.m. the animal died suddenly after muscle had been removed. Necropsy was performed immediately, but nothing of significance was found. The insulin used was tested on a normal dog and was of the expected potency.

Comment. This experiment was one of the most satisfactory of the series from a technical point of view. The animal was in fair condition during the experiment. There was practically no blood in the peritoneal cavity at necropsy. The observations show definitely that in spite of the administration of more than 400 units of insulin, and 1 gram of glucose for each kilogram of body weight each hour, there was a striking fall in muscle glycogen.

Experiment 5. The abdomen of a female bull terrier weighing 10.2 kgm. was opened October 13, 1917, and a loose ligature passed around the vena cava at the usual site for a reverse Eck fistula. A loose ligature was also passed around the portal vein at the site of ligation in a true Eck fistula. November 14, the animal weighed 8.8 kgm. and had been in good condition since the last operation. Laparotomy was performed and the portal vein and vena cava completely ligated at the site of the former ligations. January 12, 1928, the animal had remained in fair condition since the last operation. The abdomen was opened and the pancreas, liver and kidneys removed. Ether was given at 10:10 a.m.; incision was made at 10:15 a.m.; the pancreas was out at 10:40 a.m.; the liver was out at 10:45 a.m.; the kidneys were out at 10:55 a.m.; the ether was off at 10:55 a.m. and the last stitch was taken at 11:05 a.m. The liver weighed 228 grams. After operation the animal weighed 7.9 kgm. The removal of the pancreas was rather difficult. The other operative procedures were uneventful. Specimens from four muscles of the right thigh were removed for muscle glycogen estimation. At 11:30 a.m. 1 gram of glucose for each kilogram of body weight was injected intravenously in a 25 per cent solution. At 12:00 m. the blood sugar was 0.296 per cent. The injection was repeated. At 12:20 p.m. 2 grams of glucose for each kilogram of body weight was injected. At 1:30 p.m. the blood sugar was 0.536 per cent. At 1:30 p.m. 1 gram of glucose for each kilogram of body weight and 300 units of a 20-unit insulin were injected.

For two hours following the administration of insulin the animal appeared unwell, but it slowly recovered. At 2:00 p.m. 1 gram of glucose for each kilogram of body weight was injected. At 2:30 p.m. the injection was repeated. At 3:30 p.m. the blood sugar was 0.80 per cent; the injection was repeated. At 4:00 p.m. the injection was repeated. At 4:30 p.m. the blood sugar was 0.56 per cent. At 5:00 p.m., 5:30 p.m., 6:30 p.m. and 7:00 p.m. the injection was repeated. At 7:30 p.m. the blood sugar was 0.93 per cent.

Specimens of the four muscles of the thigh were taken under local anesthesia, and the animal was killed (table 5). Necropsy was performed immediately. About 80 cc. of blood was found in the peritoneal cavity.

Comment. Undoubtedly the injection of insulin brought about the formation of glycogen. This is confirmed by the fact that the blood sugar showed a definite fall from 0.80 per cent at 3:00 p.m. to 0.56 per cent at 4:30 p.m.

Experiment 6. November 14, 1927, after a specimen of blood had been taken from a male terrier, weighing 8 kgm., that had been fasted since November 11, and had been outside to reduce its muscle glycogen, the animal was etherized, both kidneys and the pancreas were removed, and the left lobe of the liver was removed for glycogen estimation. Finally specimens from the muscles of the left thigh were taken for glycogen estimation.

At 8:30 a.m. the blood sugar was 0.096 per cent; at 9:43 a.m. 0.5 gram of glucose for each kilogram of body weight was injected. At 10:15 a.m. the injection was repeated. At 10:44 a.m. the blood sugar was 0.388 per cent. The injection was repeated at 10:45 a.m., 11:15 a.m., 11:45 a.m., 12:15 p.m., 12:45 p.m. and 1:15 p.m. At 1:45 p.m., under procaine anesthesia, a piece of biceps femoris was removed, and the injection of glucose was repeated. At 2:14 p.m. the blood sugar was 0.400 per cent. At 2:15 p.m. 0.5 gram of glucose for each kilogram of body weight was injected. At 2:45 p.m. and 3:15 p.m. the injection was repeated. At 3:45 p.m. the blood sugar was 0.420 per cent. The injection was repeated at 3:45 p.m., 4:15 p.m. and 4:45 p.m. At 5:45 p.m. the blood sugar was 0.492 per cent; the injection was repeated. At 7:00 p.m. specimens of muscle from the left thigh were removed under local anesthesia and the animal was killed. The necropsy data were negative (table 6).

Comment. There was a questionable increase in muscle glycogen and a definite increase in liver glycogen in spite of the fact that the pancreas was removed.

Experiment 7. December 20, 1927, pancreatectomy and nephrectomy were performed and the usual specimens removed from the right thigh of a female collie, weighing 18.8 kgm., that had been fasted for forty-eight hours, during ten of which it was left outside at the temperature just below freezing. The animal made an excellent recovery and at 9:45 a.m. (one hour after pancreatectomy) the blood sugar was 0.320 per cent.

At 10:15 a.m. 0.75 gram of glucose for each kilogram of body weight in 25 per cent solution was injected. At 10:45 a.m. and 11:15 a.m. the injection was repeated. At 11:45 a.m. the blood sugar was 1.15 per cent; the injection was repeated. At 12:15 p.m. an injection of 1.50 gram of glucose for each kilogram of body weight was given. At 1:30 p.m. the injection was repeated. At 1:45 p.m. an injection of 0.75 gram of glucose for each kilogram of body weight was given. At 2:15 p.m. and 2:45 p.m. the injection was repeated. At 3:30 p.m. the blood sugar was 1.00 per cent. The injection was repeated at 3:30 p.m., 3:55 p.m., 4:30 p.m., 5:15 p.m., 5:45 p.m., 6:30 p.m., 7:00 p.m. and 7:30 p.m. At 8:00 p.m. the blood sugar was 1.48 per cent. At this time, under procaine anesthesia, the usual three muscles of the left thigh were removed and the animal was killed (table 7). The necropsy was negative.

Comment. The animal undoubtedly made glycogen although not so much as would occur in a non-diabetic animal in the presence of such hyperglycemia.

Experiment 8. January 11, 1928, the pancreas and kidneys were removed and specimens taken from five muscles of the right thigh for glycogen estimation from a female mongrel, weighing 10.8 kgm., that had previously been fasted for two days and placed outside in the cold for six hours. The animal was not given glucose for three hours after the operation to permit it to become diabetic.

At 9:30 a.m. the blood sugar was 0.117 per cent, three-quarters of an hour after pancreatectomy was performed. At 11:00 a.m. the blood sugar was 0.131 per cent. At 12:30 p.m. an injection of 1.50 grams of glucose for each kilogram of body weight was given intravenously. At 1:30 p.m. the blood sugar was 0.268 per cent; the injection was repeated. At 2:00 p.m. an injection of 0.75 gram of glucose for each kilogram of body weight was given. At 2:30 p.m. and 3:00 p.m. the injection was re-

peated. At 3:30 p.m. the blood sugar was 0.741 per cent; the injection was repeated. At 4:00 p.m. the injection was repeated. At 4:30 p.m. the blood sugar was 1.05 per cent. The injection was repeated at 4:30 p.m., 5:00 p.m., 5:30 p.m., 6:00 p.m., 6:30 p.m., 7:00 p.m. and 7:30 p.m. At 8:00 p.m. the blood sugar was 1.63 per cent;

TABLE 7

Experiment 7

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.218	0.347
Rectus femoris	0.081	0.409
	0.093	0.421
Vastus internus	0.238	0.309
	0.276	0.351

TABLE 8

Experiment 8

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.296	0.349
	0.291	
Rectus femoris	0.454	0.396
	0.454	0.455
Vastus internus	0.294	0.273
	0.209	
Gracilis	0.672	0.334
Semimembranosus	0.582	0.416

TABLE 9

Experiment 9

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.646	0.480
	0.632	
Rectus femoris	0.320	0.470
	0.361	0.463
Vastus internus	0.437	0.607
	0.440	0.638

the injection was repeated. At 8:45 p.m. an injection of 1.50 gram of glucose for each kilogram of body weight was given. At 9:30 p.m. specimens of the opposite thigh were taken under procaine anesthesia and the animal was killed (table 8). Necropsy data were negative.

Comment. The experiment showed that when precautions had been taken not to begin injecting glucose until several hours had elapsed after pancreatectomy the administration of glucose does not result in the deposition of muscle glycogen.

Experiment 9. March 6, 1928, the pancreas and the usual three specimens of muscle were removed from a female white and brown mongrel, weighing 15 kgm. After the operation the animal was allowed as much meat and pancreas as it desired. Twelve hours after operation it received a half-pint of milk and 15 grams of glucose; this was repeated on the morning after the operation. Thirty hours after the operation under procaine anesthesia the corresponding three muscles of the opposite thigh

TABLE 10

Experiment 10

MUSCLE	GLYCOGEN			
	Left	Average	Right	Average
	per cent	*per cent*	*per cent*	*per cent*
Sartorius	0.989	0.989	1.119	1.119
Rectus femoris	0.884 0.912	0.898	0.983 1.132	1.058
Vastus internus	1.062 1.155	1.108	1.650 1.270	1.460
Biceps femoris	1.350 1.570	1.460	1.758 1.495	1.626
Pectoralis major	0.938 1.029	0.984	1.204 0.883	1.043
Grand average		1.099		1.277

were removed and the animal killed (table 9). At necropsy pancreatectomy seemed to be complete and the wound in the leg did not show evidence of suppuration.

Comment. Apparently this animal made muscle glycogen. The experiment would have been more convincing if more muscles had been analyzed. The animal had not received food for four days. It had been permitted to shiver for two hours on March 2 and 3, and for twenty-four hours on March 5. The glycogen found at operation was therefore surprisingly high.

Experiment 10. November 28, 1927, a male hound, weighing 10 kgm., was fed, and under procaine anesthesia at 11:00 a.m. specimens of the various muscles studied in the hepatectomy experiments were removed for glycogen estimation. Five grams of muscle was always used and as a rule duplicate estimations were made, the exceptions being the sartorius muscles, from which a single estimation only could be

made on not quite 5 grams of muscle, and the pectoralis major from which duplicate estimations could be made using 4.2 grams of muscle for each single estimation (table 10).

Comment. The observations are in agreement with those of Cramer, who worked with the older Brücke method. His differences were as great as 40 per cent for the glycogen content of the corresponding muscles of the opposite side of the body. We believe that our figures represent the closest agreement that can be expected for samples of corresponding muscles of the opposite sides of the body when excised under our experimental conditions.

TABLE 11

Experiment 11

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.752	0.324
	0.758	0.330
Rectus femoris	0.966	0.229
	0.880	
Semimembranosus	0.210	0.503
		0.442
Vastus internus	0.604	0.460
		0.560

TABLE 12

Experiment 12

MUSCLE	GLYCOGEN AT BEGINNING OF EXPERIMENT	GLYCOGEN AT END OF EXPERIMENT
	per cent	*per cent*
Sartorius	0.220	0.407
	0.134	
Rectus femoris	0.315	0.741
	0.269	0.548
Vastus internus	0.390	0.646
	0.390	
Liver	0.273	1.200
	0.278	0.902
Biceps femoris*	0.799	0.976
	0.744	0.015

* Removed at 1:45 p.m.

Experiment 11. January 9, 1928, at 9:20 a.m. the abdominal content of a male mongrel, weighing 13.1 kgm., was manipulated with the hand under ether anesthesia, about as much as would occur in the removal of the liver. The abdomen was closed in the usual manner and specimens from the muscles of the left thigh were removed for glycogen estimation. The animal was put in its cage and nothing was done to it until 7:00 p.m. when the corresponding muscles were removed. The animal was then killed. Necropsy data were negative (table 11).

Comment. This mock operation showed that the glycogen of the muscle diminishes markedly when a normal dog is subjected to the procedures employed in our experiments. It would seem that when the glycogen content is unchanged at the end of an experiment, there has either resulted

the formation of a small amount of glycogen, or else the glucose has spared the breakdown of this substance.

Experiment 12. November 14, 1927, nephrectomy was performed and the left lobe of the liver removed for glycogen estimation from a female terrior, weighing 515 kgm., that had been fasted for three days and placed outside the kennels for a few hours each day to be exercised and subjected to the cool weather. Specimens from the usual three muscles of the right thigh were then secured. At 9:15 a.m. when the last stitch was being inserted the animal was given 0.5 gram of glucose for each kilogram of body weight.

At 9:45 a.m. injection of 0.50 gram of glucose for each kilogram of body weight was given. At 10:15 a.m. the injection was repeated. At 10:45 a.m. the blood sugar was 0.129 per cent; the injection was repeated. At 11:15 a.m. and 11:45 a.m. the injection was repeated. At 12:05 p.m. the blood sugar was 0.132 per cent; the injection was repeated. At 12:45 p.m. and 1:15 p.m. the injection was repeated. At 1:45 p.m., under procaine anesthesia, a specimen of biceps femoris muscle was removed. At 2:05 p.m. the injection was repeated. At 2:45 p.m. the blood sugar was 0.192 per cent; the injection was repeated. At 3:15 p.m. the injection was repeated. At 3:45 p.m. the blood sugar was 0.106 per cent; the injection was repeated. At 4:15 p.m. and 5:15 p.m. the injection was repeated. At 5:45 p.m. the blood sugar was 0.157 per cent; the injection was repeated. At 6:45 p.m., under local anesthesia, a sample of muscle was removed. The animal was killed and a piece of liver removed for glycogen estimation (table 12). Necropsy data were negative.

Comment. The experiment showed definitely that the administration of 1 gram of glucose for each kilogram of body weight each hour to a normal dog will more than double the glycogen content of the body under the conditions of the experiments in which, following hepatectomy and pancreatectomy, the administration of glucose did not cause increase in the muscle glycogen. The comparatively low blood sugars are noteworthy.

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THE CATALASE OF MALIGNANT TISSUE[1]

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Received for publication October 20, 1928

The investigations of Warburg and his co-workers on the glycolytic activity of malignant tissue have stimulated a renewed interest in the study of the general metabolism of malignant tissue as compared with that of normal tissue. Therefore, when it was seen from the publications of Michaelis, Sorensen, and Morgulis that the catalase resists a wide range of hydrogen-ion concentration, comparable to that to which the tumor virus is resistant, and that the catalase is destroyed by an excess of hydrogen peroxide, as is also the tumor virus, it was decided to undertake a study of the catalytic activity of tumor tissue and of the effect of different amounts of hydrogen peroxide upon the activity of the virus.

Most of the observations on the catalase of malignant growths were made upon the infectious tumor of the chicken, because an extract of this tissue such as one prepares for a study of the catalase contains also the tumor-producing principles, and thus the influence of hydrogen peroxide on the tumor virus could be determined in the solution used to measure the activity of the catalytic ferment.

In addition to the experiments on the chicken tumor a few observations were made on the Walker sarcoma no. 1 of the white rat and on a malignant adeno-carcinoma of the white rat.[2]

The chicken tumor used was started in this laboratory in 1925 by the injection of tumor powder[3] into the breast of a chicken. It has been passed from one fowl to another by means of tumor extract or transplants and has retained its malignancy, forming metastases and bringing about the death of the host within three or four weeks.

The amount of catalase present in the tumor was compared with that present in one or more kinds of tissue (kidney, spleen, liver, blood and muscle) from the same animal, as well as that present in the tissues of normal animals.

[1] Aided by a grant from the Bache Fund.

[2] The rat sarcoma and the rat carcinoma were obtained from Dr. George Walker.

[3] The tumor powder was obtained from Dr. James B. Murphy of the Rockefeller Institute.

In this series of experiments on the catalase content of various tissues an apparatus resembling that described by Hawk, and used by a number of investigators, was employed. It consisted of a one-arm Erlenmeyer flask and a delivery tube placed in the mouth of an inverted, water-filled 100 cc. cylinder. Four cubic centimeters of the tissue extract were placed in the flask; 5 cc. of hydrogen peroxide in a glass vial were carefully lowered into the flask, which was then tightly stoppered so as to make a closed system. The vial was upset and the flask shaken until no more oxygen was given off. When more hydrogen peroxide or more tissue extract was added to the original amount, a dropping funnel was used, and in calculating the amount of gas given off, allowance was made for that displaced by the extra fluid introduced. All the experiments were conducted at room temperature. Ordinary tap water was used in the cylinder in which the oxygen was collected and as the cylinder was filled frequently during the experiments the temperature of the water was somewhat lower than that of the extract or of the hydrogen peroxide.

The tissues were removed aseptically, weighed and ground up with glass particles. Ten times their weight of a 0.9 per cent sodium chloride solution was added and the mixture was then centrifuged for five minutes. The hydrogen-ion concentration of such a tumor extract was usually about 6.6.

Oakland dioxygen, neutral to Congo red, was used, as acid hydrogen peroxide is known to prevent or retard the action of the catalase of some tissues. The amount of oxygen given off was measured at one-minute intervals and the results were based upon the amount of oxygen given off after two and three minutes. In practically every experiment all the oxygen was given off during the first three minutes, but where a longer time was necessary to accomplish this the length of the interval was also recorded in the tables.

The infectious tumor of the chicken consistently showed very little catalytic activity. No difference was noted between the tissues of animals killed with ether and those that were bled. The thirteen tumor extracts tested gave an average of 1.38 cc. of oxygen for the period of three minutes. In several instances the tumor tissue was cut up into small particles with a pair of scissors and a 1:10 suspension made of the cells themselves in 0.9 per cent sodium chloride solution. The catalytic activity of these cell suspensions[4] was slightly greater than that of the tumor extract, for their average was 2.66 cc. of oxygen for the three-minute interval.

[4] These cell suspensions necessarily contained some red blood cells.

		cc. extract		Amount of oxygen cc.
Tumor	Chicken no.	81	plus 5 cc. H_2O_2	4
		88R	plus 5 cc. H_2O_2	1
		88L	plus 5 cc. H_2O_2	1
		47	plus 5 cc. H_2O_2	1
		45	plus 5 cc. H_2O_2	1
(Kept in ice-box 24 hrs.)		45	plus 5 cc. H_2O_2	3
		94	plus 5 cc. H_2O_2	4
		92	plus 5 cc. H_2O_2	1
		48	plus 5 cc. H_2O_2	1
(Bloody part of tumor)		109	plus 5 cc. H_2O_2	1
(White part of tumor)		109	plus 5 cc. H_2O_2	0
		132	plus 5 cc. H_2O_2	0
(Bloody part of tumor)		173	plus 5 cc. H_2O_2	0
(White part of tumor)		173	plus 5 cc. H_2O_2	0
Tumor cells[4]		47	plus 5 cc. H_2O_2	2
		45	plus 5 cc. H_2O_2	4
		94	plus 5 cc. H_2O_2	2

The sarcoma and carcinoma of the rat showed greater catalytic activity than the chicken tumor. The carcinoma was more active than the sarcoma. It was difficult to determine whether this was entirely due to a more active catalytic principle of the malignant cells or to the presence of contaminating organisms as all of the malignant growths of the rat contained areas of necrosis which may have contained some organisms. The carcinomata examined were particularly necrotic.

	¼ cc. extract		Amount of oxygen cc.
Sarcoma rat	No. 1	plus 5 cc. H_2O_2	7
	2	plus 5 cc. H_2O_2	9
	3	plus 5 cc. H_2O_2	7
Benign adenoma rat	1	plus 5 cc. H_2O_2	1
Carcinoma rat	1	plus 5 cc. H_2O_2	15
	2	plus 5 cc. H_2O_2	21
	3	plus 5 cc. H_2O_2	20

In comparing the catalytic activity of the chicken tumor with that of the normal muscle of the fowl, breast muscle from the unaffected side was usually used, although the muscle of two normal chickens was also tested. The muscle showed a lack of catalase and gave off only 1 cc. of oxygen. When equal parts of tumor extract and muscle extract were used there was no increase in the catalytic activity. This was also true when 4 cc. of muscle extract were added to tumor extract that had already been shaken up with the hydrogen peroxide. This would seem to indicate that the muscle extract neither contained an active catalytic enzyme nor activated that of the tumor.

Chicken:

2 cc. extract tumor plus 2 cc. extract normal muscle plus 5 cc. H_2O_2....0 oxygen
4 cc. extract tumor plus 5 cc. H_2O_2 plus 4 cc. muscle extract....0 oxygen

The spleen of the chicken did not show as much catalytic activity as the spleen of most animals.

		4 cc. extract		*Amount of oxygen cc.*
Spleen	Chicken no.	68	plus 5 cc. H_2O_2	14
		47	plus 5 cc. H_2O_2	28
		45	plus 5 cc. H_2O_2	14
(Kept in ice-box 24 hrs.)		45	plus 5 cc. H_2O_2	10
Spleen	Rat no.	1	plus 5 cc. H_2O_2	66
		2	plus 5 cc. H_2O_2	59
		3	plus 5 cc. H_2O_2	62
		4	plus 5 cc. H_2O_2	57
Spleen	Guinea pig no.	1	plus 5 cc. H_2O_2	65
		2	plus 5 cc. H_2O_2	61

The kidney of both the chicken and the rat showed the greatest catalase content of any of the tissues tested, and this accords with the results of recent investigators. The reaction was extremely rapid and usually was completed within the two-minute interval.

		4 cc. extract		*Amount of oxygen cc.*
Kidney	Chicken no.	68	plus 5 cc. H_2O_2	76
		88	plus 5 cc. H_2O_2	68
		47	plus 5 cc. H_2O_2	58
		45	plus 5 cc. H_2O_2	71
(Kept in ice-box 24 hrs.)		45	plus 5 cc. H_2O_2	71
Kidney	Rat no.	1	plus 5 cc. H_2O_2	65.5
		2	plus 5 cc. H_2O_2	60
		3	plus 5 cc. H_2O_2	71

Some of the chicken tumors were of the exceedingly hemorrhagic type, having only a thin rim of firm tumor tissue with the whole central mass composed of blood cells and clot while in others most of the tumor was firm white tissue. In the hemorrhagic type there was little difference in the catalytic reaction of the outer white part and the inner hemorrhagic portion. The tumors varied in amount of catalase regardless of their content of blood as for instance the tumor of chicken 173, which was largely hemorrhagic, failed to give off any oxygen even after three minutes, while the tumor of chicken 81, which gave off the most oxygen of any of the chicken tumors, was composed mostly of firm white tissue.

It was interesting to note the catalytic activity of the blood of the tumor chicken in comparison with that of clots of blood in the tumor. Whole blood showed greater activity than the red blood corpuscles from centri-

fuged blood, while in both the catalytic activity was greater than in blood clots from the chicken tumors. According to Winternitz (1909), the stroma of the red blood cells contains the catalase and post mortem clots have less catalase, due to the uneven distribution of the cells.

4 cc. extract:

	Amount of oxygen cc.
Whole blood chicken, plus 5 cc. H_2O_2	61
Centrifuged red blood cells, plus 5 cc. H_2O_2	44
Blood clot from tumor, plus 5 cc. H_2O_2	16
Blood clot from tumor, plus 5 cc. H_2O_2	10

We determined to test whether, with 4 cc. of chicken tumor extract and the corresponding 5 cc. of hydrogen peroxide, all of the peroxide was used up, or whether the reaction stopped because the catalase had been exhausted. To this end, 4 cc. of kidney extract were added to the previously mixed H_2O_2 and tumor extract. The reaction again took place but at a slower rate than when kidney extract alone was used. The amount of oxygen given off in three minutes was less than for the kidney extract, but when allowed to continue as long as ten minutes, approximately the same amount of oxygen was in two instances liberated from the 5 cc. of hydrogen peroxide. This was true when kidney extract was added to both the tumor extract and tumor cells which had previously reacted with hydrogen peroxide. Such an experiment was also done by adding muscle extract to tumor extract and hydrogen peroxide, but in these experiments no oxygen was given off.

		Oxygen cc.
4 cc. tumor extract plus 5 cc. H_2O_2	3 min.	1
plus 4 cc. kidney extract	3 min.	32
	10 min.	57
4 cc. tumor cells plus 5 cc. H_2O_2	4 min.	3
plus 4 cc. kidney extract	3 min.	32
	7 min.	47
4 cc. tumor extract plus 5 cc. H_2O_2	3 min.	0
plus 4 cc. muscle extract	3 min.	0
4 cc. tumor extract plus 5 cc. H_2O_2	3 min.	0
plus 4 cc. muscle extract	3 min.	0

The above experiments showed that the catalase of the tumor extract and cells rather than the hydrogen peroxide was entirely used up.

We were interested to see whether the tumor extract would bring about any inactivation of the kidney extract when the two were brought together in equal amounts, as the apparent lack of catalase in the chicken tumor might, it was thought, be due to an anticatalytic substance. Two cubic centimeters of kidney extract and 2 cc. of tumor extract were mixed and 5

cc. of hydrogen peroxide added. The oxygen given off was practically equal to that given off by 2 cc. of kidney extract alone. This showed that there was no anticatalytic substance in the chicken tumor.

Chicken:

		Oxygen cc.
2 cc. kidney extract and 2 cc. tumor extract	2 min.	61
	3 min.	71
	4 min.	74
Rat:		
2 cc. spleen extract and 2 cc. carcinoma extract	2 min.	23
	3 min.	33
	4 min.	40
	8 min.	62
2 cc. kidney extract and 2 cc. carcinoma extract	1 min.	59
	2 min.	64

The influence of hydrogen peroxide on the tumor virus. The addition of hydrogen peroxide to the tumor extract was followed by so little frothing that it was decided to test whether the virus remained active in such mixtures. It was soon found that there was no relation between the amount of oxygen given off by the mixture and the activity of the virus, since all such mixtures failed to produce a tumor when injected into the breast of a chicken, regardless of whether the catalytic activity had been negative or whether oxygen to the amount of 1 to 4 cc. had been given off.

A number of experiments were then undertaken to determine the minimum amount of hydrogen peroxide necessary to inactivate the tumor virus.

NUMBER OF CHICKENS INOCULATED	AMOUNT OF HYDROGEN PEROXIDE	GROWTH OF TUMOR
	per cent	
1	75	0
1	50	0
3	25	0
3	10	0
2	5	2
2	2	2
2	1	2

From these it is seen that the addition of hydrogen peroxide in amounts equivalent to 75, 50, 25 and 10 per cent inactivated the virus, while the addition of less hydrogen peroxide than this did not prevent the virus from bringing about the growth of a tumor when the mixture was injected into the breast of a chicken.

Since so little measurable oxygen was set free in the mixture of hydrogen peroxide and tumor extract, an effort was made to ascertain whether the

inactivation of the virus could be due to some other cause, such as the presence of acetanilid in the hydrogen peroxide or to a change in hydrogen-ion concentration brought about by the addition of the hydrogen peroxide. Neither of these factors seemed to be accountable. One milligram of pure acetanilid[5] added to 5 cc. of tumor extract did not bring about the inactivation of the tumor virus, and the hydrogen-ion concentration of the mixtures of tumor extract and hydrogen peroxide, which was between 5.8 and 6.2 (depending upon the amount of hydrogen peroxide added) could hardly have been injurious to the virus, since Lewis and Michaelis (1928) have shown that the virus is resistant to a range of hydrogen-ion concentration between 4 and 12.

When pure oxygen was bubbled through the tumor extract it apparently had no effect upon the activity of the virus, for tumor extracts through which oxygen had been bubbled for 15 minutes, 45 minutes, 60 minutes and even 2 hours, all gave rise to malignant growths.

Discussion. W. C. M. Lewis (1927), discussing the physico-chemical and biochemical aspects of malignant neoplasms, reviews the results obtained from investigations on the various enzymes found in malignant growths, but he fails to discuss the presence or absence of catalase in such tissue. The only reference to the catalase in malignant tissue we have been able to find in the literature, is a simple statement by Buxton (1903) that all the tumors investigated decomposed H_2O_2 with release of oxygen. Practically all investigations on the activity of the catalase, with the exception of a few observations on aerobic and anaerobic organisms by Stern (1927), have been made upon normal tissue.

The amount of tissue and of hydrogen peroxide have differed so greatly in the various experiments that have been carried on that it is difficult to compare the results obtained. However, all investigators seem to be in agreement that the relative order of the tissues in respect to enzyme content is as follows: liver, kidney, blood, lung, muscle, brain. Embryonic tissues are said to contain less catalase than those of the adult (Battelli and Stern, 1905; Buxton and Shaffer, 1905; Herlitzka, 1907). The embryonic tissue is more sensitive to the presence of acid in commercial hydrogen peroxide than is the adult tissue (Mendel and Leavenworth, 1908).

W. E. Burge (1916) seems to think that the amount of catalase present in muscle depends to some extent upon the work done by the muscle, the heart having the most active catalase, the legs next, and the breast least. By decreasing or increasing the amount of work done by the muscle one could bring about a corresponding decrease or increase in the catalase.

Burge and Burge (1921) found by experiments on the Colorado potato beetle that whatever increased the oxidation in the body increased the

[5] Obtained through the kindness of Doctor Read of Sharp & Dohme Company, Baltimore, Maryland.

output of catalase. The unfertilized ovum showed less liberated oxygen than the fertilized ovum. The amount of oxygen liberated increased from larval stage to adult and decreased in old bugs. These investigators also found that the spermatozoön furnished a substance which stimulated the eggs to increased catalase formation and that when the amines, alkalies, acetates, etc. (which Loeb found increased oxidation and brought about parthenogenesis) were introduced into the alimentary tract they caused an increase in output of catalase from the liver.

Winternitz and Rogers (1910) found that the entire fresh egg had slight catalytic power; the entire unfertilized incubated egg had no catalytic activity, but after fertilization the germinal portion of the incubated egg rapidly acquires catalytic activity, although the white and yolk show no increase.

Bodine (1921) finds that grasshoppers, fireflies and potato beetles increase in body weight with increasing age but that the amount of catalase decreases. That this is not dependent upon the fact that the amount of carbon dioxide output also decreases is shown by hibernating animals, which give off little carbon dioxide, but in these there is no decrease of catalase.

Michaelis and Pechstein (1913) show that the catalase of the liver is an ampholyt with an acid dissociation constant of $2.88–10^{-5}$ and an isolectric point of $4.31–10^{-6}$.

Morgulis, Berber and Rabkin (1926) have shown that increasing the experimental temperature causes reduction in catalytic activity, which is greater the higher the concentration of hydrogen peroxide. These investigators have found that the catalase is resistant to about the same range of hydrogen-ion concentration as Lewis and Michaelis have shown for the chicken tumor virus itself; that is, it shows a strong activity at hydrogen-ion concentrations ranging from 5 to 9. Its activity is lost entirely at pH 2 and at pH 14.

Russell and Gye (1920) found that in malignant growths in the mouse the rate of oxygen consumption rises with increasing rapidity of growth and, further, that more oxygen is used by tumors that are more highly differentiated histologically. For this reason we thought that possibly the tumor extract used the oxygen liberated from the H_2O_2. The addition of kidney extract to the mixture of tumor extract and hydrogen peroxide, however, seemed to indicate that the tumor extract had not split up the hydrogen peroxide which was then acted on by the catalase of the kidney extract, with the liberation of as much oxygen as though the tumor extract had been added to the hydrogen peroxide.

Hydrogen peroxide is quite disinfectant and in the usual commercial form, in which there is 3 per cent hydrogen dioxide, anthrax spores are killed within sixty minutes. The chicken tumor virus is killed within 15

minutes by a solution containing 10 per cent of the commercial hydrogen peroxide. When hydrogen peroxide is used as a disinfectant in contact with bacteria, it is usually broken down with the liberation of oxygen, so that an active bubbling takes place. When used in contact with the tumor extract, on the other hand, little bubbling took place, although the virus was inactivated by a weak solution of hydrogen peroxide in a short while.

The comparison of the extract of the chicken tumor with that of other organs of the same animal indicates that in so far as the catalase is concerned, the tumor more nearly resembles the muscle which it invades than any of the other organs, even though it is largely composed of blood cells; while in appearance, as well as growth in tissue culture, it resembles spleen rather than connective tissue or muscle.

From these experiments it is seen that the extract of chicken tumor is lacking in catalase and also depresses to some extent, although it does not prevent catalytic activity in extracts of other organs. That the hydrogen peroxide is not decomposed and the oxygen as formed taken up by the tumor extract is indicated by the fact that after all the reaction that can be brought about by the tumor extract has taken place the addition of an extract of some organ decomposes the peroxide, with the formation of about the usual amount of oxygen for that tissue.

Owing to the fact that such a small amount of hydrogen peroxide was necessary to destroy the tumor virus, an effort was made to determine, by injecting small amounts of the disinfectant into the tumor itself, whether hydrogen peroxide could inhibit the growth of the tumor cells. Unfortunately, this procedure usually resulted in terminating the life of the chicken, for the hydrogen peroxide did not bubble in the outer layer of the tumor but was absorbed into the blood vessels where oxygen bubbles were at once set free and these brought about the death of the chicken within a few minutes.

CONCLUSION

The infectious tumor of the chicken contains only a feeble catalase, in this respect being like the muscle. The tumor virus, however, is inactivated by hydrogen peroxide in amounts equivalent to 10 per cent of commercial peroxide. The rat sarcoma and carcinoma contain a more active catalase than the chicken tumor.

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THE FUNCTION OF THE FOWL'S URETERS

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Received for publication October 9, 1928

During the course of certain experiments on the renal function of the fowl, attention was attracted to the ureters, for it became evident that their movement could influence the minute to minute excretion of urine. It also appeared possible that they might play another rôle besides that of simple urine conveyance. Furthermore, experiments conducted to measure renal secretion pressure and inflow resistance could not be fully interpreted until data on the ureteral movements became available. No references on the subject were discovered, and indeed only a little work appears to exist on the ureteral function in general, it being commonly believed that this is a simple transport of urine carried out by means of peristaltic movements, these being partly automatic, partly under nervous control. Langley (1) claims this external innervation to be purely sympathetic, while others, including the most recent author, Macht (2), find that drugs having para-sympathetic activity also affect their movements. It will be remembered that the ureters of the fowl, unlike those of mammal, do not end in a renal pelvis, but by branching become continuous with the renal tubules. This makes it possible to inject substances freely into the kidney, even up to the glomerulus.

Normal movements. These may be profitably studied by merely inspecting the ureters under a good light, and it is often rendered particularly easy by the presence of solid lumps of uric acid which may be followed in their downward course. Movements are more or less continuous, and consist of peristaltic waves passing from above downward. First they are all from kidney to cloaca, never antiperistaltic, though this point will be referred to again later. Secondly, they appear to travel the whole length of the ureter. If the kidney is not secreting a very interesting observation can sometimes be made, namely, that the wave passing down seems to loosen its grip on the contents, which escape and rush backwards toward the kidney almost as if actually sucked. I have frequently watched a piece of uric acid moving up and down the same two or three centimeters of ureter for several minutes at a time. Under normal conditions the ureter appears to be in a state of tone throughout its whole length. In certain cases this appears very marked and the wave passing down under

these conditions appears more like a wave of dilatation, since it is actually seen as a swelling passing down the ureter. Under other conditions, especially when the bird has been made to excrete against pressure for a long period, the ureter becomes definitely lax, and if back-pressure is applied, dilates visibly. Under these conditions the wave is certainly of contraction. Sometimes there appear to be nodes of greater tone, there being intervening areas of relaxation. It is suggested that this is an intermediate condition between full tone and its complete absence.

METHOD. In order to study more fully these movements cannulae were placed in both ends of the ureter, and by connecting them to suitable instruments records were obtained. This method enabled one to study the effects of ureteral movements on the passage of fluids into and out of the ureters at varying pressures; also with varying fluids, without the disadvantage associated with interfering with the blood or nervous supply.

The operative technique consisted first, of placing a cannula in the lower end of the ureter by a peri-cloacal incision. Access having then been obtained to the kidney by the method previously described (3), a bristle stained with methylene blue is passed into the ureter where, with a good light, it is easily seen through the thin wall. Selecting a suitable place the ureter is most carefully incised over the tip of the bristle, the opening being made large enough for the cannula. Through this opening the bristle is pushed for two or three centimeters and the soft tip cut off. It is now quite easy to slip an L-shaped cannula, made from a lumbar puncture needle, over the bristle, and with a little gentle manoeuvering to insert it into the ureter. A fine ligature is then passed round the cannula by means of a small half-curved needle, and the cannula tied in position. This latter procedure is distinctly difficult at first, since bleeding is so readily started and vision becomes impossible. If however the needle be handled with care little or no bleeding results, and although this is always caused on actually tying the ligature it soon ceases unless a large vein has been punctured.

Both cannulae now in position the bristle is removed, and the cannulae connected to suitable reservoirs and recorders. For this purpose I have used the small Palmer piston recorders, which although clumsy are very sensitive. These are connected to the cannulae by means of water manometers, to which is attached a reservoir for varying the pressure.

Owing to the branching of the ureter the lower part of the kidney is still connected, and one must take into account changes of secretory rate in interpreting the tracings.

With such a preparation not only are the movements of the ureters readily recorded, but also their effects on the passage of fluid. If the pressure of the inflow (upper) end of the ureter is raised say to 20 cm. water pressure, fluid will immediately commence to run through into the outflow manom-

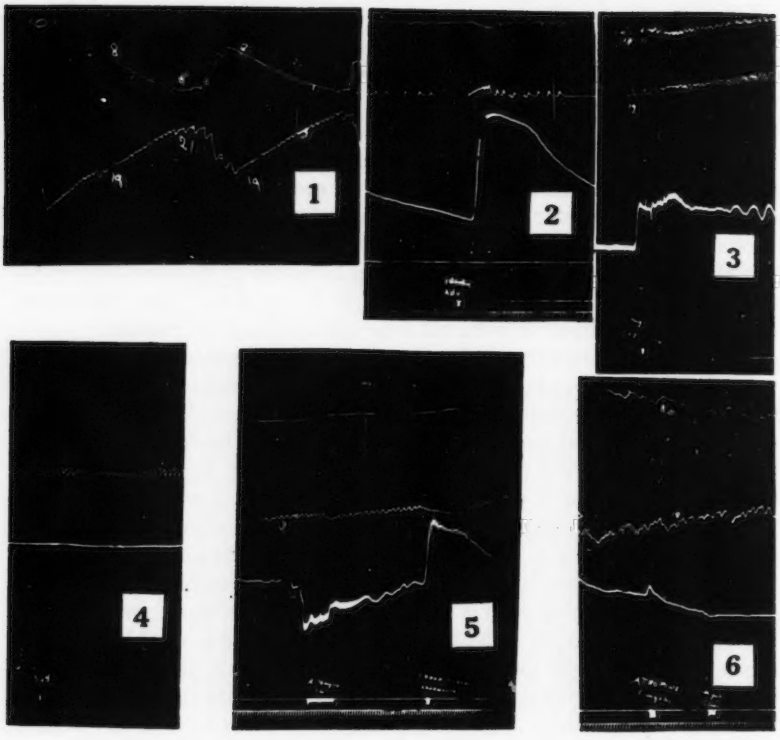

Fig. 1. Illustrates the curves produced by ureteral movements when they are "milking" effectively. Upper tracing, inflow. Lower tracing, outflow. Figures are centimeters of water. At the beginning of the tracing the levers have been moved apart. Just beyond the figures *6, 21*, will be seen the effect produced by a release of generated pressure. Original pressure in both manometers was 14 cm. water.

Fig. 2. Shows the effect of adrenalin on a quiescent ureter. Both pressures low, 3 to 5 cm. water. Notice that under these conditions the adrenalin causes rapid fine movements which are effective milking (levers come together indicating drop in inflow manometer, rise in outflow manometer). Time signal in this and other curves, 5 seconds.

Fig. 3. Shows the effect of a small dose of ergotoxin (1 mgm). Rapid fine movements, at first more effective "milking," then complicated by secretion from both ends of the ureter.

Fig. 4. Shows the effect of adrenalin following ergotoxin (20 mgm.). Inflow upper 3 cm. pressure, shows no movements, which is quite common at such low pressures. Lower outflow shows very marked adrenalin type of action. Blood pressure shows no change.

Fig. 5. Shows the effect of absence of short vagus stimulation. Notice the adrenalin type of effect at the moment of blood pressure rise, and compare with the effect of adrenalin immediately after. The ureter movements become very fine (like fig. 4) and there is an apparent inhibition of what little milking effect is taking place. Both pressures 3 cm.

Fig. 6. Shows the negative effect of atropine (1 mgm.). Stimulation of the vagus being now practically without effect. Inflow, 8.5 cm. Outflow, 18 cm. Curves coming together showing active "milking effect." (Blood pressure curve falling owing to a previous injection of adrenalin.)

eter. Soon the two pressures are equalized and in certain cases it will be seen that the flow still continues so that the fluid is passed down the ureter against pressure, which may be as high as 40 cm. of water.

One function of the ureter is thus definitely established, namely, that milking movements occur which are of such a character that not only is fluid carried down the ureter (fig. 1) but may actually be forced down against considerable resistance.

In order for such a mechanism to function, there must be some valve arrangement which will allow downward movements to take place, but which prevent flow backwards during the relaxation phase. That no real valve exists is readily shown by perfusing a dead ureter, or one in which these milking movements are absent, when it will be seen that fluid runs through either way with perfect ease.

It is not difficult however to account for this effective milking movement, and there exist at least two definite means whereby this is achieved. The simplest is merely to increase the number of waves passing down a ureter, for it will be readily understood that if an interval exists between one contraction and the next, fluid will be able to rush back into the ureter, whereas if the contractions follow one another rapidly enough this will not take place. A tracing under such conditions would show increase in number of contractions but decrease in size. Such a result is caused by a number of drugs, the most important being adrenalin (fig 2). Clearly, however, with this mechanism alone, differences in the inflow and outflow pressures could only be maintained by constant rapid movements. That this is not the case is shown by reference to figure 6. In this experiment the movements are slow yet they are most effective. On the other hand, in some cases in spite of rapid movements taking place, little or no difference between inflow and outflow pressure is developed or if that be present, actually diminished (fig. 5). A second mechanism must therefore exist. This however is easily accounted for since I have already described a tonic state involving the whole, or parts, of the ureter. Under this condition fluid being driven out of the ureter by peristaltic waves cannot reënter unless it has sufficient force to overcome the resistance offered by this tonic contraction. This mechanism is, I suspect, the normal. It may be noted that this tonus also offers resistance to inflow from the upper end; this, however, is less since waves passing down the ureter carry fluid with them. The suggestion exists that stretching the ureter sets up a peristaltic wave, and the records always show a larger tracing at inflow pressures of 20 cm. than at 10 cm. or less.

It requires but little thought to imagine the tone of the ureter increased until such resistance is created that it interferes with renal secretion. That such spasm may actually occur is indicated by the fact that on occasion fluid, even under relatively high pressure (20 to 30 cm. water), fails

to pass down the ureter. Inserting a bristle and gently twizzling it round often relieves this condition, though no actual obstruction is removed thereby. The same effect has also been noted with an ordinary renal secretion preparation, for passing a bristle into the ureter often starts up a secretion, again without evidence of blockage. That this effect might be in the nature of a reflex as described in the dog (Cushny, 4) is true, but in view of the fact that spasm seems undoubtedly to occur in perfused ureters, the more probable explanation is that the secretion was

TABLE 1

Figures showing uric acid concentration in ureteral urine

HOURLY SAMPLES	AMOUNT URINE	AMOUNT URIC ACID	PER CENT URIC IN URINE
	Experiment 1		
	cc.	*mgm.*	
1	4.1	49	1.0
2	6.7	114	1.7
3	5.4	115	2.1
4	3.5	88	2.5
5	3.5	179	5.1
6	2.9	65	2.2
7	2.1	65	3.1
8	6.4	125	1.9
	Experiment 2		
1	11.6	69.5	0.6
2	3.7	102	2.8
3	2.8	112	4.1
4	0.9	157	17.5 (sticky solid)
5	0.8	173	21.6 (sticky solid)
6	2.5	217	8.7
7	4.5	172	3.8
8	3.1	119	3.8

It should be noted that these are not exceptional results, though they are amongst the highest obtained.

being held up by ureteral spasm, this being relieved by passing the bristle. As will be shown elsewhere secretion pressure is low and no great amount of obstruction would be necessary to check it.

These experiments naturally suggest a new and important function for the bird's ureter, namely, as a mechanism that may actually control the rate of renal secretion. While there is no doubt as a comparison of the secretion pressure, inflow resistance, and effective milking movements readily shows, that ureteral spasm may hold up secretion, it is not possible

however at this moment to state definitely whether it be a normal mechanism, though it would appear not at all unlikely.

In this connection one may mention that Paton (5) states that the fowl's urine is very watery and suggests cloacal re-absorption as the important factor in concentration. While not questioning these results, there is also no doubt that extremely concentrated urine is excreted by the fowl's kidney. That is to say, it is thick, sticky, and contains large quantities of solid uric acid (see table 1). Either concentration takes place in the kidney or, as I have suggested elsewhere, uric acid is actively secreted and in this way obtains its concentration. The view which has been accepted by several writers (5, 6, 7) that the fowl's urine is always thin and watery, and is concentrated by the cloaca, is inaccurate in that it is only one of the several possible mechanisms, and does not take place under all conditions. Indeed in my own experience of several hundred birds, it occurs only if they have been taking large amounts of fluid, and even then bits of uric acid appear in the ureteral discharge as Paton also mentions. It is possible that anesthesia may interfere or alter water secretion, as Davis (7) suggests. Also it must be remembered that certain results were based on unanesthetized, but previously operated animals. In any case, however, whatever may be the normal method of concentration there is no question whatever that a very efficient mechanism exists for uric acid excretion apart from cloacal absorption.

A third question which arose as a result of a direct study of the ureteral movements was, whether the ureter may actually develop slight suction of the kidney. This has not been supported by experiments, since in no case has the intake fluid been below 2 to 3 cm. of pressure. The movements which merely keep the ureteral contents moving without actually expelling them may however serve to prevent uric acid deposits forming, and in this way have a very useful function.

Recorded movements in an anti-peristaltic direction are constantly shown in which the inflow pressure first drops (downward peristalsis), then rises again (release of generated pressure). There may however be a primary rise, as with the distal end, which might be true antiperistalsis, or a release effect from a more distal contraction. Finally one has to remember that the urine is secreted from a flaccid ureter at both ends and in experiments where the milking effect is absent the pressure rises equally in both manometers, but is interrupted by contraction waves. True antiperistalsis has never been recorded; fluid may pass up a ureter especially if flaccid, but the records do not show that the ureteral movements hasten its passage.

The control of ureteral movements. Some experiments were conducted with the object of discovering the factors controlling ureteral movements. As in any other viscera that has the combined property of tone and peri-

staltic movement, the results of these experiments are far from clear, and a considerable amount of work is still necessary to establish fully this controlling mechanism.

Certain gross factors exist, however. First, any procedure which kills the bird stops ureteral movements within a few seconds. That this is probably a nutrient factor is indicated by the fact that procedures that lower the blood pressure for a period of time are also apt to impair movements, and especially tone.

The contents of the ureter may also have effect since either alkaline $\frac{N}{10}$ or acid $\frac{N}{10}$ fluids promptly stop all movements, though these return on perfusion with Ringer. Furthermore solutions containing drugs may exert their specific effects on perfusion of the ureters.

Injections of drugs into the bird give varying results, and this appears to be partly due at any rate to the activity of the ureter at the given moment. Adrenalin unquestionably may initiate movements in a quiescent ureter (fig. 2) which was in this case due to violent hemorrhage caused in tying the ureteral cannula. In most cases it increases the number and rate of contractions though diminishes their recorded amplitude (figs. 3, 5). This increase may be associated with increased effective milking movements, or not. Some records show an absence or diminution of recorded movements in the inflow cannula. That these effects are real and not due to associated blood pressure changes is shown by the fact that adrenalin causes the same effects on perfusion. In some cases adrenalin is without effect.

This effect on the ureters partly explains the diuresis produced by adrenalin, and fully explains those cases in which following an injection of adrenalin, only one or two drops of urine are excreted. Since there is however, a total increase of urine in other cases adrenalin also possesses real diuretic properties (6, 8, 9). Ergotoxin in small doses (5 mgm.) has a somewhat similiar effect, though there may be marked increase in size and regularity of the movements (fig 4). Milking movements are sometimes rendered more effective. Following large doses, adrenalin may still cause quickening without any blood pressure change (fig. 3).

These results suggest a sympathetic control, but the absence of complete regularity in the results makes the presence of other factors clear.

Vagus stimulation per se produces no results apart from those associated with prolonged fall of blood pressure (fig. 5). During this stimulation a few rapid waves may be noted on the ureteral tracing. These are I think undoubtedly due to the liberation of adrenalin and correspond to the after-rise in other cases. Stimulation of the peripheral ends of the vagi cut below the heart is also without effect. Care must be taken in this

experiment to avoid either injury or stimulation of the adrenals since this is associated with adrenalin liberation.

Atropine either generally or locally is without demonstrable effect (fig. 6). Physostigmine has also not been shown to have an effect by intravenous administration.

These results do not support a parasympathetic control.

SUMMARY.

1. The ureteral movements of the bird are described; they consist of peristaltic waves travelling down the ureter, which is normally in a state of tone.

2. These ureteral movements have a downward milking effect, capable of working against considerable pressure.

3. The pressure developed by the ureteral movements is greater than the renal secretion pressure.

4. A new function for the ureter is suggested, namely, that of holding up of renal secretion.

5. Evidence so far accumulated indicates that these movements appear to be partly controlled through the sympathetic, not at all through the parasympathetic systems. This evidence is not complete.

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STUDIES IN HUMAN PHYSIOLOGY

I. The Metabolism and Body Temperature (Oral) under Basal Conditions[1]

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Received for publication August 28, 1928

This paper, together with those that are to follow in this series, will record observations made from February, 1925 to February, 1927 on five normal, human subjects, to wit:

A. B., under observation continuously during the two-year period; male; age, 34 to 35 years; height, 161 cm.; weight, 51 to 55 kgm.; occupation, instructor in medical school.

C. D., also under observation continuously during the two-year period; male; age, 28 years; height, 170 cm.; weight, 57 to 63 kgm.; occupation, also instructor in medical school.

E. F., under observation from February, 1925 to February, 1926; female; age, 30 years; height, 163 cm.; weight 53 to 55 kgm.; occupation, junior medical student.

G. H., also under observation from February, 1925 to February, 1926; female; age, 19 years; height, 163 cm.; weight, 55 to 58 kgm.; occupation, laboratory technician, in charge of hospital metabolism determinations.

K. L., under observation from February, 1926 to February, 1927; female; age, 24 years; height, 158 cm.; weight, 54 to 57 kgm.; occupation, laboratory technician doing analytical chemical work.

It may be important, in connection with a possible climatic effect on the functions that were studied, to state that the three women were native Buffalonians and have never been away from this region for any length of time; A. B., was born and spent most of his life in the Middle West; C. D., was born and spent his boyhood in Alaska, later lived in the Pacific Northwest, and he and A. B., had lived for a few years in Connecticut and Massachusetts, respectively, before coming to Buffalo; both had been living in this locality at least two years before this work was begun.

[1] An abstract of this work and other that is to follow has appeared in This Journal, 1927, lxxxi, 483.

We wish to express our thanks to the Administrative Board of the Medical School, University of Buffalo, for a grant by which it was made possible to employ the technical assistance necessary in this work.

During the progress of the work none of the subjects was away from this vicinity for longer than two weeks.

It will be seen that the group is very homogeneous as to occupational habits. This must be remembered as a defect or at least as a limiting factor when generalizing from the data; outdoor workers or, even, indoor workers with decidedly different routines might show quite different results. On the other hand there are advantages in such a selection which led to its adoption. Previous work has shown how difficult it is to get incontrovertible evidence of seasonal variations, menstrual effects, etc. It was thought that any evidence of such variations would stand out most sharply from an intensive study of homogeneous material. But there were still more compelling reasons for the particular selection used. Most of the data we wished to get relates to the basal condition. The advantage of having subjects with an intelligent understanding of the requirements for this highly artificial condition, so as to insure as nearly as possible its exact reduplication throughout the year, is too obvious to need emphasis. Nor was the achievement of basal conditions during the actual time of the determinations all that was required in a study extending over such a period of time as this. In order to be able to compare one basal period with another it is necessary to know accurately as to the diet, amount of exercise and general physical condition during the period. And finally, as any who have tried it can testify, such a study as this becomes unbelievably monotonous; so it is necessary to have subjects who are sufficiently interested in the outcome of the experiment to carry on to the bitter end with the same precautions and regularity as at the beginning. It was such matters as these, more than any others, that finally determined the selection of this group.

The primary purpose of this study was to determine what constancy or amount of variation might be expected, as regards the functions under observation, in normal persons living under normal conditions. In the first place, therefore, the subjects were, insofar as it was possible to ascertain, normal physically, in that they were enjoying good health and had been all their lives with the exception of the minor disturbances of childhood and adolescence. There was no serious sickness during the period of this work and had been none for many years in any case. And, secondly, the results are typical of normal living, with these two possible exceptions: no excessive physical exercise was ever to be indulged in on the day preceding the tests (this was a matter, that, due to the habits of the subjects, turned out to be of no possible significance); an excess of protein food was to be avoided on the day preceding the tests; to this end, meat was avoided entirely on this day with but a very few exceptions, and then it was eaten very moderately. In addition to this, there are very few instances in which the subject failed to get a reasonably good night's rest—six to eight hours sleep—preceding a determination.

ROUTINE OF THE DETERMINATIONS. Most of the observations and, particularly, those in this first paper, were made under strictly basal conditions; all of them were made in the fore-noon and the majority of them between eight and ten o'clock. More particularly, each subject had a specified time that was departed from only rarely. The subject, having eaten nothing since the evening before, came to the laboratory with as little delay after getting up in the morning as possible, and without any breakfast. K. L. (the woman subject during the second year) had about an hour's work to do after reaching the laboratory before she could lie down; this work was not vigorous and consisted of standing and walking about the laboratory in ordinary laboratory work. With this exception, the subjects lay down as soon as they got to the laboratory.

The preliminary rest period, in the beginning, was half an hour. This lasted until June of the first year. There was no evidence that the metabolism failed to reach a basal level in this time, but the pulse and respiration indicated that a longer period would be safter. During the last half of the first year and throughout the second the preliminary rest period was, therefore, extended to forty-five or sixty minutes.

At the end of this time the mouth temperature was taken with an ordinary clinical thermometer; precaution was used to have the bulb of the thermometer well under the tongue and it was read repeatedly until the readings checked; very often, and especially if the reading seemed abnormal, the thermometer was shaken down and a new determination made. The thermometer was calibrated at the beginning and the same one was used throughout the two years; so the readings are of absolute and, more especially, of relative accuracy.

Immediately after taking the temperature, the metabolism was determined. This was done by the Tissot, open-circuit method. The subject inhaled outdoor air which was brought to the bed through a two-inch pipe. This pipe was about fifteen feet long so the air probably reached the subject at room temperature. Saad valves were used to separate the inspired and expired air. The "T"-piece which connected the subject with the valves was longer than it should have been during the first year; it, together with the mouth-piece, provided a "dead-space" of approximately sixty cubic centimeters; during the second year this was cut down to as little as possible, perhaps five cubic centimeters. This difference had no effect on the metabolism, in so far as could be determined (see, also, 3); but it did have a most pronounced effect on the respiration, as will be shown in a later paper. The subject was connected with the system by mouth and the nose closed with a clip. The expired air was collected in a 100-liter spirometer with all of the usual precautions to secure a representative sample. The periods were always ten minutes in length. From February to June of the first year (1925) duplicate analyses were never run; there-

after the determinations were made in duplicate almost invariably on A. B., C. D., and K. L., and whenever time permitted with E. F., and G. H.

Samples of air for analysis were collected over mercury and analyzed in a Haldane type of analyser. There is no case in which the analyses were not completed on the same day as the collection of the samples; they were made in duplicate and repeated unless the customary checks were obtained. During the first year the analyzers were checked with outdoor air only occasionally and, particularly, whenever refilled or greased; during the second year they were tested regularly once a week and oftener when time permitted. A battery of four analyzers was used, with a motor driven attachment, as described by Boothby, to raise and lower the levelling bulbs. The duplicate analyses of a gas sample were always made in different analyzers and every reading was repeated until checked. The work was done with the consciousness that gas analysis is the most vulnerable point of any investigation in which it plays a part and no pains were spared to make this part of the work as accurate as possible.

Accuracy of the technique. It is admittedly unfortunate that alcohol checks were never run with this apparatus. The work does have the sanction, however, of another form of calibration, which, though less rigid, is still of considerable reassurance. In the spring of 1921 subject A. B. had his metabolic rate determined by Dr. T. M. Carpenter of the Carnegie Nutrition Laboratory, Boston. At that time his oxygen consumption was 179 cc. per minute, or 1241 calories per day. His average for the two years recorded in this work is 177 cc. of oxygen per minute, or 1227 calories per day. The agreement is very satisfactory.

Determination of the nitrogen metabolism. During the first year (1925–1926) each subject collected a twenty-four hour urine on the day preceding the metabolism determination. During the second year (1926–1927) the bladder was emptied just before lying down preparatory for the metabolism determination and again at the end of it, the time being accurately noted and the amount of urine secreted in this interval being kept for analysis. In either case the total nitrogen was determined by the Kjeldahl method.

The room temperature. The temperature to which a subject is exposed during a metabolism determination is of such direct and immediate significance that it deserves to be explicitly defined. All of our determinations were made with the subjects lightly clothed according to their customary indoor habit and either with or without such additional covering as might be necessary for perfect comfort and relaxation. In addition an effort was made to keep the room temperature as constant as possible, not only during any single determination but also from day to day, and in the neighborhood of 25°C. Actually, the room temperatures, in the re-

grettable absence of proper automatic regulation, varied as follows: extremes, 20.1°–28.0°C.; 13.5 per cent of the observations were made at temperatures between 20.1° and 24.0°; the bulk of them, 61.5 per cent, between 24.0° and 26.0°; the remainder, 25 per cent, between 26.0° and 28.0°; the mean for the entire series is 24.7°C. It is believed, however, that there was no instance in which the subject, lightly clothed, was not perfectly relaxed and comfortable either without or with the help of suitable covering.

RESULTS. I. *Body weight.* The body weight was taken, stripped, in conjunction with nearly every metabolism determination during the first year (1925) and approximately once a week or with about every other metabolism determination during the second year (1926). The monthly averages are given in table 1 and are shown graphically in part VIII of figure 3.

The definite, positive, inter-individual correlation between weight and oxygen consumption is well known (12); for the members of this group the average values for the entire period each subject was under observation are:

	Weight *kgm.*	Oxygen consumption *cc./min.*
Men:		
A. B.	52.9	177
C. D.	60.1	200
Women:		
E. F.	53.4	186
G. H.	55.6	188
K. L.	55.5	181

The correlation for the men is positive; that for the women figures out to be -0.320 ± 0.350, or practically zero, since the coefficient is even less than its probable error. When it is remembered that these averages for the women are based on a large number of determinations in each case and therefore must represent with considerable accuracy the functions involved, it is difficult to account for the lack of conformity with the general rule.

The coefficient of correlation for the entire group, on the other hand, is more in accordance with expectation, being $+0.814 \pm 0.102$.

Our principle interest in this work, however, was not to repeat or confirm what is already well known in regard to inter-individual variation. These data afford a means of determining whether or not the same or similar relationships obtain intra-individually.

A comparison of the variations in weight and oxygen consumption (especially total oxygen) for each subject (table 1) will show that there are instances of striking parallelism between the two functions; this is the

TABLE 1

Monthly averages

SUBJECT	DATE	WEIGHT (STRIPPED) KGM.	TEMPERATURE (ORAL) DEGREES CENTIGRADE	CARBON DIOXID, CC. PER MINUTE			OXYGEN, CC. PER MINUTE			NON-PROTEIN R.Q.	CALORIES PER SQUARE METER PER HOUR (DU BOIS FORMULA)	AVERAGE DEVIATION OF OXYGEN MEASUREMENTS FROM THE MONTHLY MEANS, PER CENT	AVERAGE DIFFERENCE (* PER CENT) OF DUPLICATE OXYGEN MEASUREMENTS FROM THEIR MEANS	NUMBER OF METABOLISM DETERMINATIONS
				Non-protein	Protein	Total	Non-protein	Protein	Total					
A. B.	1925 Feb.	50.8	36.6	121	28	149	140	34	174	0.87	33.0	1.7		3
	Mar.	50.8	36.5	120	33	153	138	41	179	0.87	33.8	2.8		4
	Apr.	50.8	36.4	121	32	153	142	40	182	0.85	34.3	2.9		4
	May	51.4	36.4	121	30	151	142	37	179	0.86	33.7	2.0		4
	June	52.5	36.6	131	28	159	149	35	184	0.88	34.6	2.0	1.6	11
	July	52.2	36.6	118	31	149	137	38	176	0.86	32.9	4.1	0.8	9
	Aug.	51.5	36.5	119	32	151	133	40	173	0.89	32.7	1.5	1.5	6
	Sept.	52.1	36.4	124	33	157	139	41	180	0.89	33.9	2.2	2.1	7
	Oct.	52.2	36.4	127	30	157	142	37	179	0.89	33.8	1.8	0.8	9
	Nov.	52.5	36.5	131	30	161	142	37	179	0.93	33.9	2.7	1.0	9
	Dec.	53.4	36.5	121	34	155	138	42	182	0.87	33.7	1.2	0.6	5
	1926 Jan.	54.0	36.6	119	31	150	145	38	182	0.83	33.4	1.9	1.0	8
	Feb.	53.8	36.6	113	32	145	138	41	177	0.82	32.5	5.1	1.3	11
	Mar.	54.1	36.5	112	32	144	134	39	173	0.84	31.8	2.2	1.0	20
	Apr.	53.1	36.6	115	34	149	138	42	180	0.83	33.2	2.7	1.5	16
	May	52.0	36.4	112	32	144	135	40	175	0.83	32.5	3.2	1.9	17
	June	52.3	36.5	121	29	150	140	36	176	0.87	32.9	1.7	1.0	18
	July	53.7	36.5	123	31	154	138	38	176	0.89	32.8	1.9	1.4	14
	Aug.	54.2	36.5	115	31	146	135	38	173	0.86	31.8	2.9	0.9	11
	Sept.	54.6	36.5	120	32	152	137	40	177	0.88	32.6	1.9	1.1	18
	Oct.	54.5	36.6	121	30	151	141	38	179	0.86	32.8	3.0	1.4	16
	Nov.	55.0	36.6	120	30	150	139	38	177	0.87	32.4	1.5	1.1	18
	Dec.	55.2	36.6	116	32	148	140	39	179	0.83	32.5	4.0	2.1	14
	1927 Jan.	54.8	36.5	116	32	148	138	40	177	0.84	32.3	2.0	2.1	8
C. D.	1925 Feb.	58.8	36.5	147	24	166	163	30	192	0.89	33.1	0.5		2
	Mar.	58.5	36.3	145	28	173	163	35	198	0.89	34.2	2.0		5
	Apr.	57.4	36.2	127	34	161	147	43	190	0.86	32.9	3.1		4
	May	57.6	36.2	135	31	166	155	39	194	0.87	33.6	1.7		4
	June	58.1	36.2	132	35	167	151	44	195	0.88	33.6	1.9	0.9	10
	July	58.4	36.4	136	36	172	156	44	200	0.88	34.4	2.0	0.8	8
	Aug.	58.0	36.3	131	32	163	156	40	195	0.84	33.5	2.4	0.5	5
	Sept.	58.6	36.4	132	35	167	152	43	194	0.87	33.4	2.0	1.2	9
	Oct.	58.2	36.5	128	39	167	157	48	205	0.82	34.9	1.2	1.1	7
	Nov.	59.0	36.3	133	38	171	152	48	199	0.87	34.2	1.3	0.7	7
	Dec.	59.4	36.2	134	36	170	157	46	203	0.85	34.5	2.6	1.2	7
	1926 Jan.	60.6	36.3	131	40	171	151	50	201	0.87	34.0	2.5	0.7	7
	Feb.	60.9	36.1	125	40	165	153	50	203	0.82	33.9	3.1	1.4	10

TABLE 1—*Continued*

SUBJECT	DATE	WEIGHT (STRIPPED) KGM.	TEMPERATURE (ORAL) DEGREES CENTIGRADE	CARBON DIOXID, CC. PER MINUTE			OXYGEN, CC. PER MINUTE			NON-PROTEIN R.Q.	CALORIES PER SQUARE METER PER HOUR (DU BOIS FORMULA)	AVERAGE DEVIATION OF OXYGEN MEASUREMENTS FROM THE MONTHLY MEANS, PER CENT	AVERAGE DIFFERENCE (± PER CENT) OF DUPLICATE OXYGEN MEASUREMENTS FROM THEIR MEANS	NUMBER OF METABOLISM DETERMINATIONS
				Non-protein	Protein	Total	Non-protein	Protein	Total					
C. D.	1926 Mar.	61.4	36.1	133	35	168	156	43	200	0.85	33.5	2.5	1.2	18
	Apr.	61.8	36.0	126	34	160	151	43	194	0.83	32.2	1.7	1.2	18
	May	62.2	35.9	130	33	163	156	42	197	0.83	32.9	2.4	1.3	16
	June	62.4	36.1	135	31	166	161	38	199	0.84	33.2	2.1	0.8	17
	July	61.8	36.2	133	28	161	163	35	198	0.82	33.0	1.9	1.2	14
	Aug.	61.2	36.1	131	33	164	161	42	203	0.81	33.8	3.2	2.2	10
	Sept.	61.1	36.1	140	30	170	168	37	205	0.83	34.6	1.5	0.8	14
	Oct.	60.7	36.2	133	32	165	161	40	201	0.82	33.8	1.8	1.4	16
	Nov.	61.3	36.2	135	33	168	162	41	203	0.83	34.0	2.8	1.2	16
	Dec.	61.4	36.1	136	33	169	166	41	207	0.82	34.6	1.9	1.1	14
	1927 Jan.	61.3	36.1	121	39	160	152	49	201	0.80	33.2	3.0	2.7	8
E. F.	1925 Feb.	54.1	36.4	132	25	157	162	31	192	0.82	34.9	2.5		4
	Mar.	54.9	36.2	129	29	158	155	35	190	0.84	34.3	2.5		4
	Apr.	54.4	36.4	136	25	161	162	31	192	0.84	34.9	1.5		5
	May	54.2	36.2	137	23	160	160	29	189	0.86	34.7	3.3		3
	June	52.8	36.3	129	20	149	162	25	187	0.80	34.2	1.9	0.8	8
	July	52.6	36.3	127	23	150	156	28	184	0.81	33.9	2.9	1.8	10
	Aug.	52.6	36.5	131	28	159	154	34	189	0.85	35.1	0.9	0.9	5
	Sept.	53.0	36.4	131	22	153	153	27	181	0.85	33.4	3.3	0.8	7
	Oct.	52.8	36.5	142	21	163	158	27	185	0.89	34.5	3.7		5
	Nov.	54.4	36.3	141	21	158	159	26	180	0.89	34.1	3.5		3
	Dec.	53.4	36.3	133	21	154	159	26	185	0.84	33.9	3.2		5
	1926 Jan.	54.2	36.3	128	24	152	154	30	183	0.83	33.4	2.3		4
	Feb.	53.7	36.5			153			188	0.83	35.2	1.6		2
G. H.	1925 Feb.	58.0	36.4	145	21	166	175	26	201	0.84	35.7	3.2		2
	Mar.	57.9	36.7	140	25	164	171	31	202	0.81	35.7	1.1		4
	Apr.	58.2	36.5	125	28	153	154	34	189	0.81	33.2	2.0		4
	May	57.1	36.5	148	20	168	172	24	197	0.86	35.3	1.2		5
	June	55.9	36.5	135	18	153	163	22	185	0.83	33.4	2.1	1.9	7
	July	55.2	36.6	136	23	159	157	29	186	0.87	33.9	2.9	1.9	8
	Aug.	54.9	36.6	125	26	151	148	32	180	0.85	32.6	2.4		3
	Sept.	54.8	36.1	136	19	155	159	24	183	0.85	33.3	3.2		4
	Oct.	54.5	36.5	139	20	159	161	24	185	0.87	33.9	3.4		4
	Nov.	54.5	36.4	129	25	154	153	32	184	0.85	33.5	3.3		2
	Dec.	55.1	36.6	131	27	158	158	34	192	0.83	34.6	1.8		4
	1926 Jan.	55.3	36.6	122	24	146	150	30	179	0.82	32.2	2.8	0.6	6
	Feb.	54.7	36.6	131	21	152	165	25	190	0.80	34.4	6.6		2

TABLE 1—*Concluded*

SUBJECT	DATE	WEIGHT (STRIPPED) KGM.	TEMPERATURE (ORAL) DEGREES CENTIGRADE	CARBON DIOXID, CC. PER MINUTE			OXYGEN, CC. PER MINUTE			NON-PROTEIN R.Q.	CALORIES PER SQUARE METER PER HOUR (DU BOIS FORMULA)	AVERAGE DEVIATION OF OXYGEN MEASUREMENTS FROM THE MONTHLY MEANS, PER CENT	AVERAGE DIFFERENCE (± PER CENT) OF DUPLICATE OXYGEN MEASUREMENTS FROM THEIR MEANS	NUMBER OF METABOLISM DETERMINATIONS
				Non-protein	Protein	Total	Non-protein	Protein	Total					
K. L.	1926 Feb.	57.3	36.7	113	29	142	152	36	188	0.74	34.2	4.2	1.9	6
	Mar.	57.0	36.8	110	34	144	148	42	190	0.75	33.6	2.7	1.1	18
	Apr.	56.5	36.8	116	29	145	153	35	188	0.76	33.9	1.6	1.0	18
	May	56.4	36.8	112	27	139	148	34	182	0.76	32.6	2.8	1.2	16
	June	56.2	36.8	109	32	141	141	40	181	0.78	32.6	2.6	0.6	18
	July	54.8	36.9	107	31	138	144	38	182	0.75	33.1	1.6	0.7	12
	Aug.	54.6	37.0	111	25	136	144	31	175	0.77	32.0	2.3	1.1	10
	Sept.	54.3	36.9	108	25	133	143	31	174	0.76	31.7	2.5	0.8	16
	Oct.	54.4	36.9	107	26	133	143	32	175	0.75	32.0	1.6	0.9	11
	Nov.	54.8	37.1	112	24	136	146	30	176	0.77	32.2	2.8	0.8	17
	Dec.	54.9	37.0	115	24	139	150	31	181	0.77	33.0	4.3	1.1	13
	1927 Jan.	54.7	37.1	118	22	140	160	28	187	0.74	34.2	3.5	1.5	6

testimony, also, of the average curves for weight and total oxygen (fig. 3). Just how close this parallelism is in the day-to-day fluctuations of each individual is shown in the following tabulations which give the coefficients of correlation for the weight, on the one hand, and the (1) total and (2) protein oxygen, on the other:

Coefficients of correlation for day-to-day, intra-individual variations in body weight and

1. Total oxygen consumption

A. B.	-0.0451 ± 0.0713 (89)*
C. D.	$+0.3631 \pm 0.0628$ (87)
E. F.	$+0.0971 \pm 0.1139$ (36)
G. H.	$+0.5572 \pm 0.0718$ (42)
K. L.	$+0.4758 \pm 0.0796$ (43)

2. Protein oxygen†

	(1925)	(1926)
A. B.	-0.0126 ± 0.0984	$+0.1336 \pm 0.1035$
C. D.	$+0.4282 \pm 0.0830$	-0.2424 ± 0.0925
E. F.	$+0.0088 \pm 0.1124$	
G. H.	$+0.1115 \pm 0.1040$	
K. L.		$+0.4798 \pm 0.0801$

* (Number of observations.)

† (The calculations for the protein oxygen were made separately for the two years because of the different methods of urine collection that were used.)

These results are at once intriguing and disappointing. In the cases of C. D., G. H. and K. L. the correlation between the weight and oxygen consumption is high enough to be of significance and to lead to the hope that here might be a partial explanation of the day-to-day variations in the metabolic rate. It is difficult to take this suggestion seriously, however, in the face of the total lack of any demonstrable correlation between these two variables in the cases of A. B. and E. F.

And, finally, even in those cases which show a significant, positive correlation between the day-to-day variations in weight and oxygen consumption, this correlation is only about half as great as that which is known to exist between these two variables inter-individually.

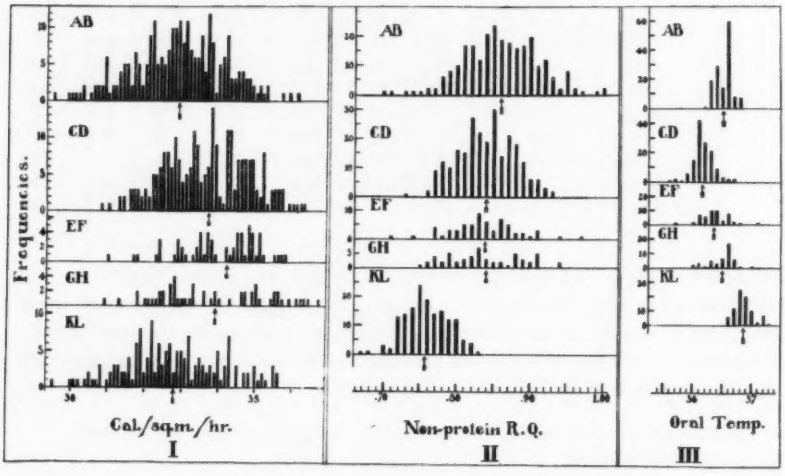

Fig. 1. Frequency polygons for I, calories per square meter per hour; II, non-protein respiratory quotient; III, oral temperature. In each case the arrow indicates the arithmetical mean.

II. Oral temperature. *1. Individuality.* Figure 1, part III, shows the statistical distribution of the temperature readings for each subject; the statistical constants are given in the last item of table 2. From these it is seen that only two of the subjects, A. B. and G. H., have approximately the same mean temperatures, which are 36.52° and 36.50°C., respectively. The other means are: C. D., 36.17°; E. F., 36.35°; K. L., 36.87°. These differences are much greater than the probable errors and appear not to be due to error of sampling, for each distribution seems statistically complete.

2. Seasonal variation. Figure 3, part VII, shows the monthly averages for each subject (from table 1) and (lowest curve) the grand average for each month.

In each of our cases the body temperature reaches a low point during, or remains low throughout the spring; and the typical sequence seems to be a rise throughout the summer and fall. The curve for C. D. illustrates this most clearly, although it is complicated by a curious and unexplainable difference between the mean temperatures for each year (36.29° for 1925; 36.10° for 1926). There are serious enough departures from such a scheme (e.g., A. B., July–December, 1925; and G. H., August–December, 1925) to prevent it from being taken as conclusively proved by these data; nevertheless there is sufficient agreement, especially during the second year (A. B., C. D. and K. L., 1926) to make it a reasonable inference and to justify further attention being given to it.

3. Menstrual variation. By averaging the temperature readings made on the women during the menstrual periods and during the first, second, third and fourth weeks following, the figures given in the last item of table 3 were obtained; these are shown graphically in the last column of figure 2. From this evidence it seems possible to conclude without any doubt that there is a rise in body temperature preceding the onset of menstruation. Whether the lowest point occurs during or just after the period is less certain; two of the three subjects show the lowest point following, rather than during the period; and the remaining subject (G. H.) is so irregular that the average curve probably loses rather than gains in accuracy by the inclusion of her data. The range of this variation is uncertain in the case of G. H., but approximates 0.25 of a degree Centigrade, with E. F. and K. L.

4. *Correlation of body (oral) temperature with the oxygen consumption:* Although the mouth temperature could not be taken during the actual collection of the expired air for the metabolism determination, it was taken so shortly before that there is no good reason to believe it is not indicative of the actual body temperature at the time of the measurement of its oxygen consumption.

The coefficient of correlation of the two variables for each subject is as follows:

A. B.	$+0.022 \pm 0.040$	(260)*
C. D.	$+0.010 \pm 0.043$	(246)
E. F.	$+0.016 \pm 0.084$	(65)
G. H.	$+0.006 \pm 0.091$	(55)
K. L.	-0.009 ± 0.050	(162)

* (Number of observations.)

Not only are the coefficients small, but in no case are they as large as the probable error; in other words, there is no demonstrable correlation between the intra-individual variations of oral temperature and oxygen consumption under strictly basal conditions.

Although this seems to be the necessary conclusion in regard to the intra-individual variations in temperature and metabolism, there is a possibility of a definite, inter-individual relationship as shown in the following table:

	Mean oral temperature °C.	Mean oxygen consumption cc./min.	Mean heat production cal./sq.m./hr.
Male			
A. B.	36.52	177	32.9
C. D.	36.17	200	33.7
Female			
E. F.	36.35	186	34.2
G. H.	36.50	188	33.9
K. L.	36.87	181	32.8

Here is the rather surprising suggestion of a negative correlation between the mouth temperature and the metabolic rate. For the group as a whole the correlation is as follows:

Temperature × Oxygen consumption	-0.695 ± 0.156
Temperature × Cal./sq.m./hour	-0.648 ± 0.179

The correlation becomes much more apparent, however, when the sexes are considered separately and the metabolic rate is expressed as calories per square meter per hour. Neither of these limitations is unwarranted for it is well enough known that the inter-individual comparisons of metabolic rate must take account of sex and size. When this is done, for the members of this group, it is interesting to find that the three temperature-heat-production points for the women fall on the same straight line and those for the men on another line below but almost exactly parallel with that of the women; and both of the lines have a negative slope. It may, perhaps, be premature to attach too much importance to a result observed on only five individuals; yet, when it is remembered that each of these averages is based on from 50 to 260 observations, more than a casual significance may be seen in this very definite negative correlation between body temperature and heat production among the members of this group.

III. Basal metabolism. The primary purpose of this work was to collect data which might throw some light on the vexed question of seasonal variation. Quite naturally, however, the results are related to other problems of human metabolism and some of these may be mentioned before coming to the matter of chief interest.

1. Statistics: A. Statistical distribution and constants of the data. The number of practically consecutive observations on single individuals which were made in the course of this work gives interest to a statistical analysis of the results as a further contribution to the study of intra-individual variation (1) (2) (3). In addition to the total oxygen consumption and heat production, which have been the measurements principally analyzed previously, information will be given here in regard to the carbon dioxid

TABLE 2

Statistical constants

FUNCTION	SUBJECT	MAXIMUM AND MINIMUM	"MODE"*	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER OF OBSERVATIONS
Total oxygen, cc. per minute....	A. B.						
	1925	162–192	182	179.5 ±0.6	7.70	4.29	79
	1926	161–195	176	176.3 ±0.2	5.14	2.91	181
	Total	161–195	176	177.3 ±0.3	6.51	3.67	260
	C. D.						
	1925	183–211	192–198–203	197.7 ±0.5	6.34	3.21	75
	1926	185–219	199	200.6 ±0.4	6.87	3.43	171
	Total	183–219	203	199.7 ±0.3	6.81	3.41	246
	E. F.	169–201	188–190	186.3 ±0.6	7.19	3.86	65
	G. H.	170–207	184	188.1 ±0.9	9.66	5.13	55
	K. L.	162–203	173–183–186	181.4 ±0.4	8.24	4.54	162
Non-protein oxygen, cc. per minute.....	A. B.						
	1925	127–163	141	140.9 ±0.6	7.87	5.59	79
	1926	115–162	141	137.5 ±0.4	7.40	5.38	179
	Total	115–163	141	138.5 ±0.3	7.56	5.46	258
	C. D.						
	1925	140–174	155	154.1 ±0.6	6.97	4.52	74
	1926	130–183	154–162–169	159.4 ±0.5	9.36	5.87	171
	Total	130–183	153–157–160	157.8 ±0.5	8.67	5.49	245
	E. F.	136–174	157	157.5 ±0.7	7.72	4.90	63
	G. H.	137–182	158	163.3 ±1.1	12.26	7.51	55
	K. L.	125–167	142	146.7 ±0.5	8.73	5.95	156
Protein oxygen, cc. per minute.	A. B.						
	1925	29–51	39–41	38.2 ±0.5	4.78	12.49	48
	1926	29–53	36	38.9 ±0.4	5.11	13.13	89
	Total	29–53	36	38.7 ±0.3	4.98	12.86	137
	C. D.						
	1925	29–57	41–43–45–47–49	43.0 ±0.6	6.17	14.35	47
	1926	28–67	43	41.2 ±0.5	6.58	15.96	85
	Total	28–67	43	41.8 ±0.4	6.49	15.52	132
	E. F.	19–38	25	29.1 ±0.4	4.49	15.46	48
	G. H.	13–42	25	28.1 ±0.7	6.92	24.63	46
	K. L.	9–53	40	36.2 ±0.7	9.86	27.25	80
Calories per square meter per hour.....	A. B.						
	Total	29.5–36.1	33.7	32.86 ±0.05	1.21	3.69	260
	C. D.						
	Total	30.8–36.3	33.8	33.65 ±0.05	1.14	3.39	246
	E. F.	31.0–36.6	34.8	34.18 ±0.11	1.31	3.84	63
	G. H.	30.9–36.7	32.8	33.86 ±0.13	1.47	4.34	55
	K. L.	29.5–35.6	32.2	32.77 ±0.07	1.29	3.92	157

* This use of "mode" is not strictly accurate for the values have not been computed from the fitted frequency curve; it is merely used to designate the measurement having the greatest, actual frequency.

TABLE 2—*Continued*

FUNCTION	SUBJECT	MAXIMUM AND MINIMUM	"MODE"*	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER OF OBSERVATIONS
Total carbon dioxid, cc. per minute	A. B.						
	1925	139–175	159	154.5 ±0.8	9.83	6.36	79
	1926	131–177	151	148.4 ±0.4	7.31	4.92	181
	Total	131–177	153–154	150.3 ±0.3	7.89	5.25	260
	C. D.						
	1925	152–181	167–173	168.1 ±0.5	6.89	4.10	75
	1926	148–188	170	165.1 ±0.4	7.03	4.26	171
	Total	148–188	170	166.1 ±0.3	7.07	4.26	246
	E. F.	138–178	157	155.0 ±0.7	8.02	5.18	65
	G. H.	138–179	150–169	156.6 ±0.9	9.90	6.32	55
	K. L.	120–156	138	139.5 ±0.4	7.22	5.18	164
Non-protein carbon dioxid, cc. per minute	A. B.						
	1925	104–144	120–127–129	123.7 ±0.7	9.87	7.25	77
	1926	92–143	116–117	117.0 ±0.4	8.68	7.42	179
	Total	92–144	117	119.0 ±0.4	9.24	7.76	256
	C. D.						
	1925	115–156	131	133.1 ±0.6	8.07	6.06	74
	1926	107–154	130	132.1 ±0.5	8.86	6.71	171
	Total	107–156	130	132.4 ±0.4	8.61	6.51	245
	E. F.	109–156	129	131.7 ±0.7	8.66	6.58	63
	G. H.	113–159	143	133.9 ±1.0	11.38	8.66	55
	K. L.	94–134	112	111.3 ±0.4	7.48	6.72	157
Protein carbon dioxid, cc. per minute	A. B.						
	1925	23–41	32	30.8 ±0.4	3.87	12.55	48
	1926	23–43	31	31.4 ±0.3	4.12	13.13	89
	Total	23–43	31	31.2 ±0.2	4.03	12.92	137
	C. D.						
	1925	23–46	36	34.6 ±0.5	5.04	14.55	47
	1926	22–54	34	33.1 ±0.4	5.32	16.08	85
	Total	22–54	33	33.6 ±0.3	5.25	15.60	132
	E. F.	16–30	22	23.5 ±0.4	3.69	15.71	48
	G. H.	11–34	22	22.7 ±0.6	5.63	24.84	46
	K. L.	8–42	32	27.8 ±0.6	7.31	26.27	80
Non-protein R.Q.	A. B.						
	1925	0.78–1.00	0.84–0.90	0.877±0.005	0.06	7.26	79
	1926	0.70–1.00	0.85	0.851±0.003	0.05	5.72	179
	Total	0.70–1.00	0.85	0.859±0.002	0.05	6.04	258
	C. D.						
	1925	0.78–0.93	0.85–0.87–0.88	0.863±0.004	0.05	5.44	74
	1926	0.73–0.91	0.85	0.829±0.002	0.04	4.23	171
	Total	0.73–0.93	0.85	0.839±0.002	0.04	4.58	245
	E. F.	0.71–0.97	0.83	0.837±0.004	0.05	5.56	63
	G. H.	0.75–0.94	0.83	0.840±0.005	0.05	6.14	55
	K. L.	0.70–0.83	0.75	0.758±0.002	0.03	4.29	156

TABLE 2—*Concluded*

FUNCTION	SUBJECT	MAXIMUM AND MINIMUM	"MODE"	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER OF OBSERVATIONS
Oral temperature 0°C.....	A. B.						
	1925	36.3–36.8	36.6	36.50 ±0.015	0.15	0.41	48
	1926	36.2–36.8	36.6	36.53 ±0.009	0.13	0.36	90
	Total	36.2–36.8	36.6	36.52 ±0.006	0.14	0.38	138
	C. D.						
	1925	35.9–36.7	36.3	36.29 ±0.031	0.32	0.87	47
	1926	35.6–36.4	36.1	36.10 ±0.010	0.14	0.38	84
	Total	35.6–36.7	36.1	36.17 ±0.012	0.20	0.55	131
	E. F.	35.7–37.1	36.3–36.4	36.35 ±0.022	0.23	0.64	50
	G. H.	36.0–37.0	36.6	36.50 ±0.021	0.21	0.58	46
	K. L.	36.6–37.3	36.8	36.87 ±0.012	0.16	0.44	83

production and, also, the protein and non-protein fractions of the metabolism which, so far as we know, have not been treated from this point of view before.

The incidence of the observations on the quantitative and qualitative metabolism, i.e., the calories per square meter per hour (Du Bois formula) and the non-protein respiratory quotient, is shown in figure 1, parts I and II, respectively. Calories per square meter per hour were used for this graphic representation, rather than the oxygen consumption, because of the closer correspondence in this unit of the measurements on the different subjects. The oxygen consumption gives closely similar frequency polygons; and it can be seen in table 2 that the most significant measure of variability, the coefficient of variation, is very similar for the two, being only slightly higher in two of the subjects for the oxygen consumption than after correction for differences in the respiratory quotient and body weight. The average coefficient of variation for the five subjects is exactly the same, 3.83, for both oxygen consumption and calories per square meter per hour.

The carbon dioxid production, on the other hand, has a much higher variability (average coefficient of variation for the five subjects, 5.09) than the oxygen consumption (3.83), a fact which has long been known, although an exact measure of it in statistical units has not been at hand.

A very interesting difference is seen in the relative variability of the protein and non-protein fractions of the metabolism. The coefficient of variation for the protein metabolism (average for the five subjects; either as protein oxygen or carbon dioxid) is 16.85; this is decidedly greater than that for either the non-protein oxygen (5.63) or carbon dioxid (7.07).

The following tabulation makes evident another point of much interest

and importance in connection with the variability of the different fractions of the metabolism:

	AVERAGE COEFFICIENT OF VARIATION	
	Women	Men
Calories per square meter per hour	4.03	3.54
Total oxygen	4.51	3.48
Total carbon dioxid	5.56	4.86
Non-protein oxygen	6.12	5.38
Non-protein carbon dioxid	7.32	6.95
Protein (oxygen or carbon dioxid)	22.45	14.05

In every instance the average for the women indicates greater variability than that for the men so that there is evidently a sex difference in the variability of the metabolism. This is undoubtedly confirmatory evidence of a disturbing effect of menstruation, the particular nature of which will be dealt with more precisely in a later section.

Another point of some interest in connection with the protein metabolism is shown by the data of A. B., and C. D., for the two years, 1925 and 1926, respectively. It will be recalled that during the first year the protein metabolism was calcuated from nitrogen determinations on twenty-four hour urines; during the second year the urine excreted during the one-and-a-half or two-hour period in which the subject was reclining for the metabolism determination was used. It is therefore interesting to note that in spite of this difference in the method of urine collection the maxima and minima, the modes, the means and the coefficients of variation are practically the same for both years with each of these subjects (who were the only ones subjected to both methods of urine collection).

Correlation of the total and the protein oxygen: One of the reasons originally in mind for determining the protein metabolism separately was to see whether or not the day-to-day variations in total nitrogen excretion, i.e., protein breakdown, might be the cause or might bear any significant relation to variations in the total oxygen consumption. In the meantime, Wishart has published (13) the results of work of his own on this same subject which would lead one to expect a higher degree of correlation than our own data seem to justify; they are as follows:

Coefficients of correlation for the protein and total oxygen

	1925	1926
A. B.	+0.10 ±0.10 (48)*	+0.19 ±0.07 (89)*
C. D.	+0.37 ±0.08 (47)	+0.17 ±0.07 (85)
E. F.	+0.18 ±0.09 (48)	
G. H.	+0.01 ±0.10 (46)	
K. L.		+0.31 ±0.07 (80)

* The number of observations.

All of the coefficients are positive but only two of them, C. D., 1925, and K. L., are of any significance statistically. It may be recalled in this connection that these subjects were not on any sort of a fixed or controlled diet. The primary purpose of this study was to establish the conditions of normal, every-day living. Therefore there were no stipulations as to kind or quantity of food; the only requirement was that no meat, i.e., no excess of protein food should be eaten on the day preceding the metabolism determination. It was during this day, ending the morning of the metabolism determination, that the twenty-four-hour urine was collected on which the total nitrogen determinations (for the calculation of the protein oxygen) were made during 1925. Although this seems to give a higher correlation, in the case of C. D., than when the urine was collected during the metabolism period (1926), not much can be made of this because the evidence from all of the other subjects shows that the method of urine collection was not important in this connection. And, on the whole, the only conclusion that these data permit is that there is no constant or significant correlation between the total nitrogen excretion and the total oxygen consumption.

The respiratory quotient: In nearly every case the respiratory quotient shows a higher variability than the quantitative metabolism (see table 2), the average coefficient of variability, 5.47, being higher than that for total oxygen, 3.83, or calories per square meter per hour, 3.83, or total carbon dioxide, 5.09. That is, the quality of the metabolic materials seems to be less constant than the total metabolic level.

In short-cut metabolism determinations it is customary to assume a standard respiratory quotient of 0.80 or 0.82. Inspection of these data shows that this figure is not suited to the average condition of any of these subjects. In the cases of C. D., E. F. and G. H., the mean values are, indeed, not very far from this, being in the neighborhood of 0.84; but A. B., with a mean value of 0.86 and, even more so, K. L., with a mean value of 0.76 seem to indicate that there may be an individuality in the quality of the metabolism (without any recognizable pathology) just as was shown above to be the case in connection with the body (oral) temperature. Furthermore, inspection of part II of figure 1 aids in removing any doubt that these results may be due to errors of sampling; for these frequency polygons make it very apparent that the fluctuations about the respective means constitute, in each case, a complete and unique statistical universe.

B. Variability of duplicate and day-to-day oxygen determinations. The variability which has been discussed in the preceding section is undoubtedly due, in part, to error of observation; it may presumably be contributed to, also, by actual variations in the metabolic rate. The variations in metabolism may, in turn, be of two kinds, viz., day-to-day fluctuations and periodic variations, perhaps of greater extent and seasonal in duration.

There can be no question as to the inevitability of errors of observation; and there is by this time good reason to believe in the existence of actual day-to-day variations in the metabolism; whether or not there are seasonal variations is still disputed, and certainly there has been no attempt to determine what part either of the three may contribute to the total variation.

These comparisons may easily be made with the help of the following tabulations:

SUBJECT	EXTREME DEVIATION OF		
	(1) Duplicate determinations from their means	(2) Daily determinations from the monthly means	(3) Daily determinations from the yearly means
A. B.	5.3	8.4	9.6
C. D.	4.5	7.5	9.0
E. F.	4.5	8.1	8.6
G. H.	3.0	8.0	9.8
K. L.	3.3	8.3	11.3
Average	4.1	8.1	9.7

	AVERAGE DEVIATION OF		
	(1) Duplicate determinations from their means	(2) Daily determinations from the monthly means	(3) Daily determinations from the yearly means
A. B.	1.3	2.5	2.8
C. D.	1.2	2.1	3.4
E. F.	1.0	2.5	3.0
G. H.	1.4	2.8	3.8
K. L.	1.0	2.7	3.8
Average	1.2	2.5	3.4

(All of the figures are percentages of the respective means.)

From June, 1925 on to the end of this work (February, 1927) the observations made on A. B., C. D. and K. L. were, almost without exception, made in duplicate; many of those on E. F. and G. H. were also, although lack of time prevented its being done systematically on these subjects. These data are capable of providing some information, therefore, as to the relative magnitude of the three variables mentioned above; the variation of duplicate determinations from their means will be a measure of the observational error; the deviation of daily averages from the monthly means may be taken as a measure of the day-to-day variability; and finally, if the daily fluctuations are superimposed upon larger seasonal variations this should be apparent from a comparison of the day-to-day

variability with the variability for, let us say, an entire year—as measured by the average deviation of the daily determinations from the yearly means.

In every instance, whether dealing with the extreme or average deviations, these increase in magnitude as we pass from duplicate to day-to-day determinations and then on to those made throughout the entire year. Evidently, then, the metabolism is inherently variable independently of the inescapable observational errors; and, in addition, it not only varies from day-to-day but, to a greater extent, throughout the year. This latter point will be of significance in connection with the discussion, in a later section, of the possibility and nature of seasonal variations in metabolism.

Evidently the greater part of the observed variability in metabolism is due to the combined effect in, apparently, about equal proportions, of errors of observation and day-to-day fluctuations in the metabolic rate. A further analysis of these two factors is of interest, therefore, from the point of view of the reliability and accuracy of the technique as well as for the light that may be thrown on the expected magnitude and frequency of the metabolism variations.

The following table shows the number of times (in per cent of the total number of observations for each individual, as well as, in addition, the grand total) that duplicate oxygen determinations were found to vary by as much as a certain per cent from their mean.

VARIATION OF DUPLICATE OXYGEN DETERMINATIONS FROM THEIR MEANS	PER CENT OF THE NUMBER OF OBSERVATIONS ON:			
	A. B.	C. D.	K. L.	Total
per cent				
±0.0–1.0	43.5	59.5	61.5	53.9
2.0	84.5	81.1	89.7	84.6
3.0	95.6	93.7	98.7	95.7
4.0	97.3	99.1	100.0	98.6
5.0	98.2	100.0		99.3
6.0	100.0			100.0

(E. F. and G. H. are omitted from this table because the number of duplicate determinations made on them was so small compared with the other three.)

A similar table for the variation of the day-to-day determinations from the monthly means follows:

DEVIATION OF DAY-TO-DAY DETERMINATIONS FROM THEIR MONTHLY MEANS	PER CENT OF THE NUMBER OF OBSERVATIONS ON:					
	A. B.	C. D.	E. F.	G. H.	K. L.	Total
cc. oxygen						
±0.0–5.0	71.0	72.0	63.1	63.6	63.4	68.6
10.0	94.0	93.1	95.4	94.5	91.4	93.3
15.0	100.0	100.0	100.0	100.0	100.0	100.0

The absolute value of the deviations in cubic centimeters of oxygen may be converted into percentages with sufficient accuracy by dividing with the average oxygen consumption for each subject; or even by dividing by the average for the whole group, which is 186 cc. per minute. Five, ten and fifteen cubic centimeters then become 2.7, 5.4 and 8.1 per cent, respectively.

By way of summary, and in round numbers, then, we may say that approximately 50 per cent of the duplicate determinations differed from their means by less than 1 per cent, and approximately 95 per cent of them by less than 3 per cent; and of the day-to-day determinations, over 50 per cent (68.6 per cent) differed from the monthly mean by less than 5 cc., or about 2.5 per cent, while nearly 95 per cent differed by less than 10 cc., or about 5 per cent.

And finally, the extreme deviation of 15 cc., or 7.5 to 8.4 per cent above or below the average monthly value seems to show that the customarily allowed variation of 10 per cent is a greater latitude than is necessary for repeated observations made within not longer than monthly intervals. It is only when the observational period is longer than a month (the only additional unit for which we have made the calculations is a year) that the amount of variation to be expected becomes so high (or nearly so, the average for our series being 9.7 per cent) and then only as a very exceptional, extreme variation.

C. Agreement with accepted standards. The following table summarizes the data related to this subject:

SUBJECT	SEX	AGE	WEIGHT	HEIGHT	CAL./SQ.M./HR.			CALORIES PER DAY		
					Obs.	Aub-Dubois standard	Diff.	Obs.	Harris-Benedict standard	Diff.
			kgm.	*cm.*			*per cent*			*per cent*
A. B.	M.	35	53.0	161	33.1	39.5	−16.2	1227	1371	−10.5
C. D.	M.	29	60.0	170	33.7	39.5	−14.7	1365	1547	−11.8
E. F.	F.	30	53.6	163	34.3	36.5	−6.0	1291	1329	−2.9
G. H.	F.	20	55.8	163	34.0	38.0	−10.5	1303	1396	−6.7
K. L.	F.	24	55.5	158	32.9	37.0	−11.1	1230	1366	−9.9

The Harris-Benedict standards give the better prediction, although, even they, as so much other evidence has indicated, are too high.

2. The effect of sleep on the total oxygen, total carbon dioxid and respiratory quotient. Two of the subjects, A. B. and C. D., found it very easy to go to sleep under the quiet and soothing conditions of the metabolism measurements; the other three subjects were never known to do so. Fortunately for purposes of comparison, the sleep always occurred during the first of consecutive, duplicate determinations. Ih about half of the cases the

subjects merely dozed; in the remainder they slept rather soundly throughout most of the ten-minute period. It has been impossible to show any difference between the two cases so all periods in which there was any sleep at all have been included without discrimination in the calculations. For comparison, an equal number of determinations, during both of which the subjects were wide awake, have been taken at random from approximately the same times of the year. The averages are as follows:

SUBJECT	OXYGEN (CC./MIN.)			CARBON DIOXID (CC./MIN.)			NON-PROTEIN RESPIRATORY QUOTIENT		
	1st	2nd	Diff.	1st	2nd	Diff.	1st	2nd	Diff.
A. B. (W. 20)*	177.4	174.3	−3.1	149.1	148.9	−0.2	0.869	0.868	−0.001
A. B. (S. 20)	176.8	176.9	+0.1	148.3	153.5	+5.2	0.847	0.885	+0.038
C. D. (W. 10)	201.9	201.4	−0.5	162.8	165.7	+2.9	0.807	0.828	+0.021
C. D. (S. 10)	201.9	201.7	−0.2	166.2	169.7	+3.5	0.829	0.852	+0.023

* W, awake during both periods; S, asleep during the first period; the number, 20 or 10, is the number of determinations on which each average is based.

Inspection of these data shows that in the case of C. D. the effect of sleeping during the first period was negligible. In the case of A. B., sleep apparently reduced the oxygen consumption an average of 3.2 cc. per minute, or 1.8 per cent, and the carbon dioxid excretion 5.4 cc. per minute, or 3.1 per cent below what they would have been if awake. This unequal effect on the oxygen and carbon dioxid is undoubtedly due to a pronounced effect on the volume of the respiration, as will be shown in a later paper; and as a consequence of it the respiratory quotient is lowered during the sleep period by 0.039, or 4.6 per cent.

These effects are not large; in the case of the oxygen, which is the only one for which the calculation has been made, the reduction by sleep, 1.8 per cent, is only slightly greater than the average variation of duplicate determinations from their means, which was shown above to be, for A. B., 1.3 per cent. For this reason and also because the sleep periods were rather uniformly scattered throughout the year, these periods have been included in the general statistics.

These conclusions do not apply, necessarily, to the metabolism during prolonged, deep sleep. It may be that in the ten minutes at their disposal these subjects did not reach a very deep state of slumber; or it may be that the processes involved in going to sleep and in waking up (both of which were measured in these cases) are antagonistic and algebraically add up to zero. The results show only what may be expected as a consequence of dozing during a short metabolism determination.

3. The effect of menstruation. The literature on this highly disputed subject has, fortunately, been so well reviewed recently (5) that nothing

needs to be done here about it, except to supply one omitted reference; the recent, careful work of Hitchcock and Wardwell (11) deserves to be men-

TABLE 3

The effect of menstruation; averages of the observations on the women, arranged according to their position in the menstrual cycle

The numbers in parenthesis are the numbers of observations on which each average is based.

FUNCTION	SUBJECT	MENSTRUAL PERIOD	INTER-MENSTRUAL PERIOD			
			First week	Second week	Third week	Fourth week and longer
Total oxygen, cc. per minute	E. F.	185.6 (7)	184.0 (19)	185.5 (11)	187.3 (13)	189.0 (15)
	G. H.	189.5 (8)	185.6 (15)	184.0 (11)	192.1 (12)	190.3 (9)
	K. L.	178.2 (12)	179.1 (39)	180.9 (35)	183.9 (36)	184.6 (35)
	Av.	183.4	181.7	182.4	186.3	186.6
Total carbon dioxid, cc. per minute	E. F.	156.4 (7)	154.2 (19)	151.7 (11)	154.0 (13)	158.8 (15)
	G. H.	156.5 (8)	153.7 (15)	155.5 (11)	159.8 (12)	158.3 (9)
	K. L.	135.2 (12)	138.6 (39)	137.0 (35)	140.6 (36)	142.2 (35)
	Av.	147.0	145.7	143.9	145.6	148.9
Non-protein oxygen, cc. per minute	E. F.	160.1 (7)	156.1 (18)	156.9 (11)	155.8 (13)	160.1 (14)
	G. H.	163.9 (8)	158.5 (15)	154.6 (11)	163.8 (12)	161.4 (9)
	K. L.	144.9 (10)	146.6 (39)	146.3 (34)	144.7 (36)	150.8 (33)
	Av.	155.2	151.4	150.0	150.8	154.8
Non-protein carbon dioxid, cc. per minute	E. F.	136.0 (7)	131.6 (18)	129.0 (11)	128.5 (13)	134.8 (14)
	G. H.	135.8 (8)	131.6 (15)	132.1 (11)	136.9 (12)	135.1 (9)
	K. L.	108.7 (10)	112.4 (39)	109.7 (34)	108.9 (36)	115.2 (33)
	Av.	125.0	121.2	117.9	118.7	123.3
Protein oxygen, cc. per minute	E. F.	25.5 (6)	29.3 (13)	28.8 (9)	31.3 (10)	28.8 (10)
	G. H.	25.4 (7)	28.3 (11)	29.6 (9)	27.7 (10)	28.8 (9)
	K. L.	30.4 (5)	32.6 (20)	34.5 (18)	39.3 (18)	33.2 (17)
	Av.	26.8	30.5	31.8	34.1	30.9
Non-protein, R.Q.	E. F.	0.850 (7)	0.839 (18)	0.826 (11)	0.824 (13)	0.849 (14)
	G. H.	0.829 (8)	0.831 (15)	0.853 (11)	0.839 (12)	0.839 (9)
	K. L.	0.754 (10)	0.768 (39)	0.730 (34)	0.734 (36)	0.764 (33)
	Av.	0.805	0.799	0.773	0.774	0.797
Oral temperature, 0°C	E. F.	36.35 (6)	36.18 (14)	36.44 (9)	36.39 (10)	36.45 (11)
	G. H.	36.43 (7)	36.54 (11)	36.44 (9)	36.59 (10)	36.46 (9)
	K. L.	36.82 (6)	36.82 (20)	36.76 (18)	36.91 (18)	36.98 (18)
	Av.	36.53	36.55	36.60	36.69	36.71

tioned because it is additional evidence toward the conclusion pointed to by our work and others in the literature.

The data are presented as averages of the determinations made during the periods and during the first, second, third and fourth weeks following; these are given in table 3 and figure 2. It must be emphasized that absolutely no determinations are included in these averages in which the subjects suffered the least discomfort. Several determinations were made in the course of the work in which this did occur but, as is well known now,

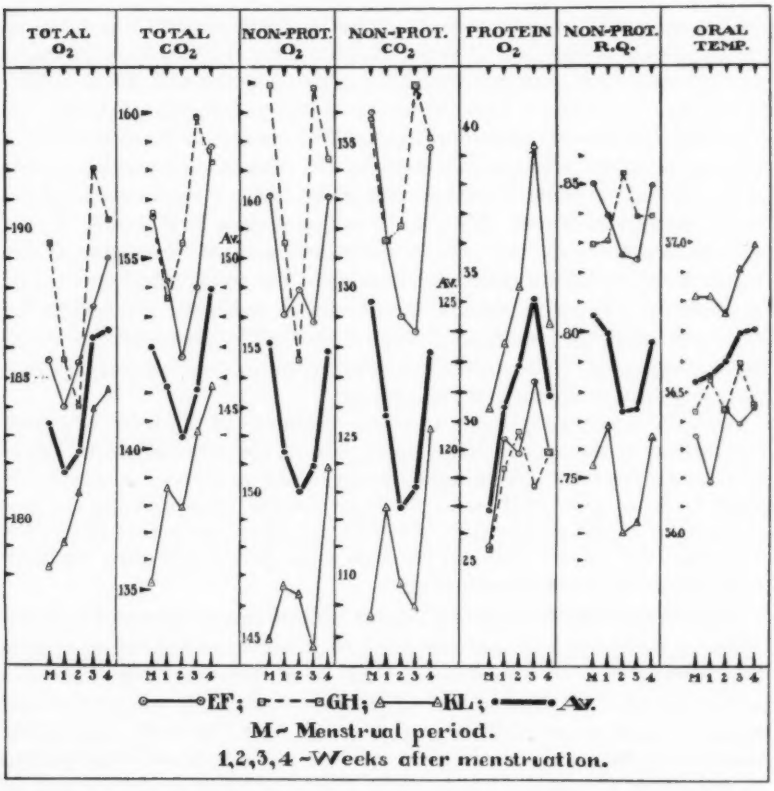

Fig. 2. The effect of menstruation

they immediately revealed themselves by their abnormally high values. The conditions of these determinations were strictly basal during as well as between the menstrual periods.

One matter in which all three of the subjects agree almost unanimously, so that the result seems rather conclusive, is the course of the nitrogen metabolism; in each case this is lowest during menstruation. In two

cases, E. F. and K. L., the highest point is reached during the third week following menstruation; with G. H. this occurs in the second week; but the depression which she shows in the third week appears as an irregularity on the curve and is perhaps not worthy of too much emphasis. The conclusion which seems warranted from these data is that the nitrogen metabolism is lowest during the period and reaches its highest value in the second or third week following.

This unanimity in the results shown by the three subjects is interesting, further, because with two of them, E. F. and G. H. (1925), the nitrogen was determined from twenty-four hour urines, whereas with K. L. (1926), the excretion during the metabolism period was used for the analysis.

Another point with respect to which all of these data agree is that the total metabolism, whether measured by the oxygen consumption or the carbon dioxid excretion, is highest during the third or fourth week following the menstrual period. Two of the subjects, again E. F. and K. L., are alike in having the highest value in the fourth week; G. H. is again exceptional, with the highest value a week earlier. But again, this disagreement is probably not too important, for the rather arbitrary division of the cycle into weekly periods is, no doubt, too rigid. The important agreement in showing a high point toward the end of the inter-menstrual period is the significant conclusion from these data.

A similar uncertainty exists as to the incidence of the point of lowest metabolism in the cycle. Here, again, two of the subjects are in agreement, with the third dissenting, although in this matter the alignment is shifted. E. F. and G. H. show the most marked depression in the first and second weeks, respectively, after the period; in the case of K. L., the metabolic rate is definitely lowest during the period and rises regularly to its maximum in the fourth week.

All of those interested in this matter will, of course, interpret this evidence in their own way and according to whatever experience or opinion they already have in respect to it. One point, however, may deserve to be mentioned. All three of the subjects were extremely coöperative and aware of their responsibilities in connection with this work; but of the three, K. L. was, perhaps, the most uniform, meticulous and conscientious in respect to all matters which might affect the basal condition; and, of still more importance and relevance in this connection, many more determinations were made of her metabolism than of either of the other two. Perhaps sufficient weight has been given to this last point, however, in making up the averages; the data are remarkably uniform in certain important particulars; where they differ, they may indicate bona-fide, individual variations, which may be the underlying cause of the confusion which characterizes this subject.

Little need be said of the non-protein metabolism; the data in the table

and the figure show the same relative differences to exist between the three subjects in this, as in the other matters just discussed; the average curves are roughly the reciprocals of the curve of protein metabolism, i.e., the lowest point is mid-way in the inter-menstrual period.

A final matter of considerable interest is the respiratory quotient. The oxygen and carbon dioxid vary similarly during the cycle, but they do not vary proportionally; and, although here again individual differences are apparent, yet the average curve shows a marked lowering during the second and third weeks of the inter-menstrual period.

The effect of menstruation on body temperature has already been mentioned.

4. The effect of vacation. The only point which we wish to discuss in this connection is the effect of the summer vacation, because it was the only period during the work which involved, at least for two of the subjects, a considerable change in habits. In each year the vacation period was the second and third weeks of August; and since there was no appreciable change in weight in any of the subjects during this time, the data may be most simply presented by tabulating the oxygen values for the month preceding (July) and the month following (September) the vacation.

SUBJECT AND YEAR	OXYGEN CONSUMPTION (CC. PER MINUTE)				
	July	September	Difference		
			Cc. per minute	Per cent	
A. B., 1925	176 (9)*	180 (7)	+4	+2.2	Ten-day canoe trip
A. B., 1926	176 (14)	177 (18)	+1	+0.6	On the beach
C. D., 1925	200 (8)	194 (9)	−6	−3.0	Ten-day canoe trip
C. D., 1926	198 (14)	205 (14)	+7	+3.4	Ten-day canoe trip
E. F., 1925	184 (10)	181 (7)	−3	−1.6	At home. No special outdoor activity
G. H., 1925	186 (8)	183 (4)	−3	−1.6	At home. No special outdoor activity
K. L., 1926	182 (12)	174 (16)	−8	−4.5	At home. No special outdoor activity
Average				−0.6	

* The number in parenthesis is the number of determinations on which the average is based.

The results entirely confirm the recent conclusion of Benedict (10) that vacations are without noticeable effect on the metabolism. In the

cases of A. B. and C. D., this is true in spite of vigorous out-door exercise with sufficient exposure, in a bathing suit, to produce a deep tan.

5. Seasonal variation. A review of this literature has only recently been given by Gustafson and Benedict (6) in the latest contribution to this subject.

Our own data are presented as monthly averages for each subject in table 1; these, together with the grand average for each month calculated therefrom, are shown graphically in figure 3.

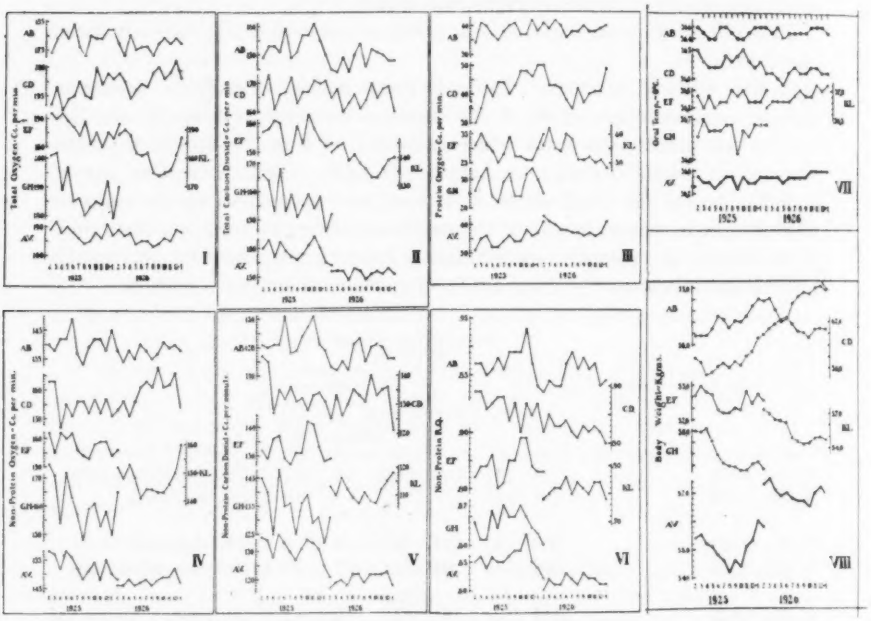

Fig. 3. Monthly and total averages for I, total oxygen; II, total carbon dioxid; III, protein oxygen; IV, non-protein oxygen; V, non-protein carbon dioxid; VI, non-protein respiratory quotient; VII, oral temperature; VIII, body weight.

A. Protein metabolism: This is shown as the protein oxygen in part III of figure 3. It may be remembered that during 1925 this datum was calculated from analyses of twenty-four hour urines; during this year there seems to be no agreement over any considerable period of time between the four subjects—a condition which is reflected in the lack of any definite seasonal swing to the average curve.

During 1926 the protein metabolism was calculated from analyses of the samples excreted during the metabolism periods, in the hope of getting a

clearer picture of the really basal protein breakdown. It has been shown that this difference in method of collection made no appreciable difference in the mean values or other statistical constants in the cases of A. B. and C. D., who were subjected to both; neither did it make any noticeable difference in the menstrual effect as shown by E. F. and G. H. (twenty-four hour samples) in comparison with K. L. (basal samples).

In connection with a possible seasonal effect, however, the method of collection does seem to be important although it is not at all clear how this could be affected without the others. Nevertheless, if the average curve for 1926 is taken as a model, it does seem possible to recognize broad resemblances between the three subjects for this year in spite of glaring discrepancies. The most serious of these is the failure of K. L. to rise during the fall and winter in correspondence with the other two; this undoubtedly makes the average curve lower than it should be at this time and obscures the incidence of the low point, which probably should be in July or August.

B. Non-protein metabolism: The curves for the non-protein oxygen and carbon dioxid are shown in parts IV and V, respectively, of figure 3; it seems quite unnecessary to undertake any detailed comparison of them. A consideration of the three curves for 1926 is all that is necessary to show that one may have anything, from no clear-cut effect at all to very pronounced cycles which are almost the exact reciprocals of each other. This lack of agreement is faithfully reflected in the average curves, which are completely negative as far as evidence for a seasonal variation is concerned.

C. Total carbon dioxid: The curves are given in part II of figure 3. Here there appears to be somewhat more uniformity and there is an evident depression in the records of all of the subjects during the summer of 1925; this is borne out by the smoothness and regularity of the average curve for this year. All of the subjects of this year, however, show a sharp decline which, beginning in the fall or early winter, promises, in the cases of E. F. and G. H., and does actually, with A. B. and C. D., reach a minimum in the spring of the following year. During this second year, also, K. L. shows a gratifyingly smooth and uniform variation which, however, is quite unlike that of either of the other two subjects during the same period and reaches its lowest point in the latter part of the fall. This lack of uniformity during 1926 is revealed in the average curve for this year which fails to show any characteristic seasonal effect. In this function, therefore, there is evidence suited to please everybody, except those who believe that the metabolism is lowest during the winter.

In concluding the discussion of the evidence up to this point, it is fitting to call attention to the fact, which can hardly have escaped notice, that there has been no sufficient concurrence or uniformity of result among the different subjects to give average curves which are at all alike for the two

years; or for that matter, granting that the incidence of the climatic effects might be slightly different in the different years, of average curves which show a definite seasonal cycle for *both* years. This latter point would seem to be almost crucial; and it is one which has not been met by any other attempted solution of this question. This digression is inserted at this place in order to better emphasize, by way of contrast, the approximate fulfillment of this test by the two functions that remain to be noticed.

D. The total oxygen consumption: The curves are shown in part I of figure 3; they are all sufficiently alike to produce relatively smooth and almost identically similar average curves for *both* years. These average results show a very uniform variation in the oxygen consumption, with minimum values for both years in August and maxima in the winter or early spring. Perhaps it is not wise to be too critical of such gratifying results; and at the time of our preliminary publication they were accepted enthusiastically at their face value. Since then, however, the individual differences have steadily assumed increasing significance; C. D., for instance, does not fit into such a scheme at all; although his curves do show more or less of a depression during the summer of each year, the lowest points are unmistakably and very symmetrically in April of both years. A. B. has values for February, 1925 and March, 1926 which are as low as those for August. And, although the women fail to show this spring depression as evidently as the men, they are not in agreement as to the incidence of the minimum; these are (if the apparently anomalous low value for G. H., in January, 1926 may be omitted) in August, for G. H.; September, for K. L.; and November, for E. V.

These discrepancies persist after correction of these figures for changes in body weight, i.e., the curves for calories per square meter (Du Bois formula) are, with very minor exceptions, the duplicates of these for oxygen consumption. The only difference, insofar as the incidence of the maxima and minima is concerned, is that the low point for E. F., in November, is eliminated. This simplifies matters somewhat for we then have A. B. and G. H., with minima in August; and E. F. and K. L., with minima, and C. D. with, at least, a marked depression in 1925, during September.

In one other respect the calories per square meter differ from the raw figures for oxygen consumption, viz., in this unit the metabolic rates of the women return to the same level at the end as they had at the beginning of their respective years; instead of showing a slight, steady rise throughout the two years, the metabolic rate of C. D. is practically uniform; and that of A. B., instead of remaining almost level, shows a very slight decline throughout the two year period.

The extent to which the highest and lowest monthly values depart from the yearly mean is given, as percentages of the latter, in the following table:

	Oxygen Diff. from yearly mean *per cent*	
A. B.	−2.83	+2.26
C. D.	−2.76	+3.52
E. F.	−3.22	+3.22
G. H.	−7.44	+4.25
K. L.	−4.97	+3.87
Average	−4.24	+3.42

E. Respiratory quotient: This function, like the total oxygen consumption and heat production shows sufficient uniformity to give average curves of some similarity for both years; these are shown in part VI of figure 3. In all cases, with the exception of C. D., who is entirely unique, there is an increase in the respiratory quotient throughout the year to a maximum in the summer or fall. The incidence of the maximum effect, however, is quite unlike in the two years; in 1925 it comes late in the fall; in 1926 it is in the early summer; and finally, the extreme nonconformity of C. D. to either of these schemes must be patiently remembered.

The extent to which the maximum and minimum monthly values are less than or exceed the yearly mean are shown as percentages of the latter in the following table:

	Respiratory quotient Diff. from yearly mean *per cent*	
A. B.	−3.4	+4.6
C. D.		
E. F.	−4.7	+5.9
G. H.	−4.7	+3.5
K. L.	−2.6	+2.6
Average	−3.8	+4.2

F. Summary of seasonal variation: Our evidence gives reason to believe that there may be seasonal variation in the total and protein oxygen and respiratory quotient; and to these must be added the body weight and temperature which were discussed in previous sections. In connection with the two latter it will be recalled that there proved to be no correlation between temperature and metabolism, intra-individually; and although three of the subjects did show significant correlation between the variations in weight and oxygen consumption, the average coefficient of correlation for the entire group was brought so low, 0.29 for the total and 0.18 for the protein oxygen, by the other two that a causal relationship can hardly be looked for here. What, then, may be considered the cause of the apparently seasonal variations in metabolism?

Much has been made in the immediately preceding sections of the lack of exact correspondence among the different subjects; that is, although

each, separately, may show a clear-cut, seasonal variation the points of maximum and minimum effect are not synchronous in the different individuals. This has been emphasized because it would seem to make it clear that the results cannot be attributed, either, to the operation of some inexorable climatic factor whose incidence on all of the subjects would necessarily be simultaneous.

Rather, the variations in metabolism must be only indirectly related to the seasons, as by the quantity and quality of the food or the amount of exercise or some other factor or factors at present unknown; factors, however, which might easily have an approximate, though not necessarily an exact seasonal coincidence in the majority of individuals of any given group, or which might be quite unique in their incidence in special cases.

Such a distinction is no mere quibble. It serves to direct attention from such matters as sunlight (7), outdoor temperature (8) and "cosmic influences" (9) to the necessity for a more carefully kept physiological diary than has as yet been attempted for the requisitely long period of time.

This point of view may carry with it, also, an explanation of the conflicting reports in the literature as to the time of incidence of the maximum and minimum seasonal effects. The prevailing finding, as in our work, has been a lowered metabolism during the warmer parts of the year. When the reverse is found, as has been recently reported for a group of college women near Boston (6), to take only one instance from near at hand, the discrepancy can hardly be explained as due to widely, or even moderately different climatic conditions. Nor is the difference necessarily due to errors of technique; for Gustafson and Benedict, in the work just cited, also found that the pulse rates of their subjects were lowest in the winter; and we shall be able to show in a subsequent report that the basal pulse rates of our subjects give the most unequivocal evidence of a summer minimum. Here is a difference, corroborating the respective metabolism findings, which no one would feel inclined to attribute to errors of technique on either side.

The conclusion seems warranted, therefore, that there is not *a* seasonal variation in metabolism; but that there may be seasonal *variations* in metabolism. This implies that the climate does not affect the metabolism directly, but indirectly and by virtue of the habits and activities, which in a homogeneous group would, as an average, be approximately seasonal in their incidence.

SUMMARY

Two men were under observation over a period of two years; during this time three women were also studied for periods of one year each. During the time they were under observation there were no restrictions on the habits or activities of the subjects, except an avoidance of an excess of

protein food and muscular activity on the day preceding the tests. The results are therefore typical of normal, every-day living in normal adults of both sexes. The observations reported here, with the exception of the weight, were all made under strictly basal conditions.

The results are shown by statistical analysis to conform to the usual standard of accuracy demanded of work of this kind. They lead to the following conclusions:

1. There is no significant correlation between the intra-individual variations of temperature and metabolism, weight and metabolism or protein and total metabolism. On the other hand, there is the usual inter-individual correlation between weight and metabolism; and, in addition, these subjects show a remarkably clean-cut, negative correlation between the inter-individual values for temperature and metabolic rate.

2. Average and extreme deviations have been computed for duplicate, day-to-day and yearly determinations of the oxygen consumption in an effort to assess the part played by each in the total variability.

3. The women prove to be more variable than the men for all fractions of the metabolism.

4. Sleep, either dozing or sleeping rather soundly during a ten-minute metabolism period, had no effect with one, and a negligible effect (decrease) on the total oxygen, carbon dioxid and respiratory quotient of another subject.

5. Vacations, even when spent in such out-door activity as a ten-day canoe trip with much tanning, cannot be shown to affect the metabolism.

6. Menstruation has a decided effect on all of the functions studied; this is so concisely summarized in figure 2 that it need not be repeated here.

7. Seasonal variation: The basal oral temperature probably undergoes a seasonal variation with, in these subjects, a minimum during the spring. Neither the protein nor the non-protein fractions, taken separately, nor the carbon dioxid excretion show concurrent seasonal variations with the different subjects. The total oxygen consumption and the calories per square meter (Du Bois formula) show roughly concurrent variations in four of the subjects, with minima in the late summer or early fall; in one of these there is, also, a marked depression in the spring; and, in the fifth, this spring depression is much the most pronounced. The respiratory quotient also shows a seasonal change, being higher during the summer or fall, with four of the subjects, than during the colder parts of the year; but the fifth subject is quite anomalous, and, among the four, there is the same lack of exact correspondence which characterized the oxygen consumption.

The divergent incidence of these effects in these subjects, as well as the divergent reports in the literature, suggest the hypothesis that the metabolism is not directly affected by the climate, but is only indirectly related to it by reason of habits and activities, which, in a homogeneous group,

might be expected to have an approximate seasonal concurrence. Our notes are not full enough to suggest anything more definite by way of conclusion.

8. Standards of metabolism: The Aub-Du Bois and Harris-Benedict standards are both too high for the members of this group; of the two, the latter gives the better prediction.

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HUMAN SKIN TEMPERATURE AS AFFECTED BY MUSCULAR ACTIVITY, EXPOSURE TO COLD, AND WIND MOVEMENT

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Received for publication October 27, 1928

Under normal conditions of rest about 75 per cent of the heat lost from the body is from the skin, chiefly by radiation, and 25 per cent is by vaporization of water from the lungs and skin. Hence the measurement of skin temperature of itself becomes suggestive in studying the conditions of heat loss. Determinations of the effects of exposure to cold and of exercise upon the skin temperature are also useful, since they enable an approximate estimate of the heat lost by radiation to the environment. Thus far in physiology emphasis has been laid in large part upon rectal temperatures. In recent years, however, importance has been attached to the skin temperature as a factor which may prove of great value, not only in studies in physiology but likewise in pathology (Cobet, 1926).

In connection with a series of metabolism studies on walking (Benedict and Parmenter, 1928) with students at Mount Holyoke College, South Hadley, Massachusetts, many observations were made at essentially the same time upon the skin temperature. These observations throw light upon the effects upon skin temperature of different types of muscular activity outdoors and indoors, of exposure to cold for both short and long periods, and of wind movement.[1]

Apparatus used for the measurement of skin temperature. A simple technique for the measurement of skin temperature, based upon the well-known thermo-electric principle, has for several years been in use in the Nutrition Laboratory (Benedict, Miles and Johnson, 1919a, 1919b; Benedict, 1925). The apparatus has been described in detail, with several illustrations, by Benedict, Coropatchinsky and Finn in an article published in French (1928a) entitled "Étude sur les mesures de température de la peau," and in an article in English (1928b) entitled "The measurement of skin tem-

[1] In these measurements we were assisted by Miss Miriam F. Clarke and Miss Madeleine E. Field of Mount Holyoke College and by Miss Mary D. Finn of the Nutrition Laboratory, to whom we wish to express our appreciation for their coöperation.

perature." The apparatus[2] consists of two copper-constantan thermoelements, one of which is immersed in a bath held constant (usually in a vacuum bottle) at not far from 31°C. The other, looped much in the form of a hairpin and protected by a hard rubber holder filled with cotton-batting, is applied to the surface of the skin. The difference in electrical potential between the two junctions, as registered on a galvanometer, serves as a measure of the difference between the temperature of the skin and the temperature of the constant junction. A calibration is made by immersing the application junction in alcohol of known temperature and noting the galvanometer deflection caused by the difference in temperature between this junction and the junction in the constant temperature bath. This test gives a factor representing the equivalent in terms of temperature of each millimeter deflection of the galvanometer. When the application junction is applied to the skin, the difference in temperature between the skin and the "constant" temperature junction can be computed by multiplying the galvanometer deflection by this factor, and the true skin temperature is rapidly obtained by adding to or subtracting from the temperature of the bath, as the case may be, this computed difference in temperature. A thermos bottle may serve as a constant temperature bath, but for a long series of experiments an electrically heated and controlled thermostat is desirable.

Subjects. Twenty-six healthy, normal women served as subjects for the various experiments. Of these all but three were students at Mount Holyoke College, who volunteered for the tests and coöperated in every way.

Topography of measurements. To determine the average skin temperature is difficult. Theoretically, innumerable points on the body surface should be measured and, indeed, simultaneously. This is impracticable. We therefore selected for measurement typical points at different parts of the body. Many of these were selected because of their accessibility under the clothing, although doubtless other points could equally as well have been chosen. As the result of a long period of development of technique in the Nutrition Laboratory, arbitrary numbers have (perhaps unfortunately) been assigned to these points of measurement, and since these numbers have been consistently used in earlier publications (Benedict, Miles, and Johnson, 1919a, 1919b; Benedict, 1925; Benedict, Coropatchinsky, and Finn, 1928a, 1928b) it seems inadvisable to change the numerical arrangement. Outlines of the front and back views of the human body are given in figure 1, showing the approximate locations of the various points and the numbers arbitrarily given to them. Of these points those chiefly on the front of the body, particularly the trunk and left side, were studied in our experiments.

[2] The entire apparatus can be secured from W. E. Collins, 555 Huntington Avenue, Boston, Mass.

Skin temperature, chiefly of the extremities, after a short period of walking in the cold out-of-doors. When one has been lying for a long period, well covered with bed-clothing, the skin temperature is fairly even and in general averages not far from 33° or 34°C. This obtains, however, only, after a relatively long stay in bed. Most of the day is not spent in bed, and to ascribe a uniform skin temperature of 34°C. to the entire body (as is not infrequently done by writers) is, we believe, erroneous. The

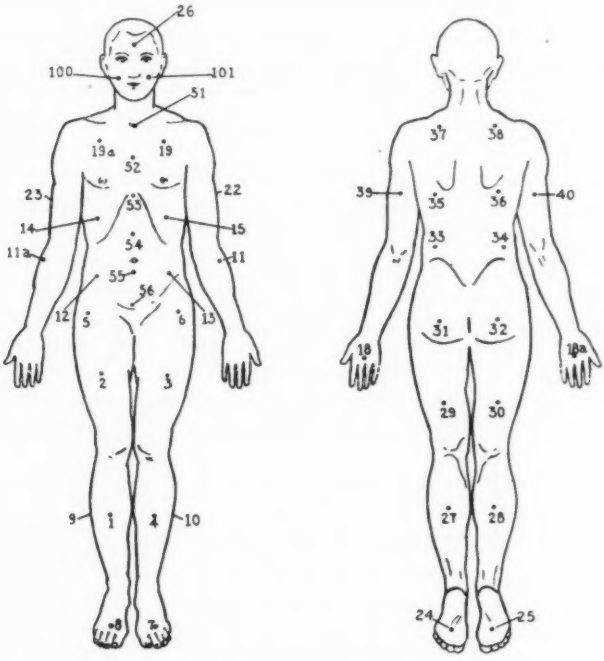

Fig. 1. Outline of the human body, front and back views, showing approximate locations of the skin-temperature measurements.

modern clothing of women, when dressed for the house, certainly affords but scanty protection. Sport clothes are somewhat heavier and undoubtedly more protective, but it is a question whether this protection is such as to maintain an equable skin temperature under conditions of outdoor activity. We felt, therefore, that a study of the skin temperature, particularly of the extremities, immediately after a short period of activity out-of-doors would give an indication of the normal temperature extremes found even upon the protected parts of the body. For this purpose 18

experiments were made on 8 young women. The subjects exercised outdoors, either between 8 and 9 a.m. or between 1:30 and 2 p.m., walking rather rapidly for from 5 to 45 minutes, usually for about 15 minutes. The clothing varied somewhat with the subjects, but was that usually worn by them in outdoor walking. The points measured after the exercise were the forehead, the left and right cheeks, one point on the left side of the back near the waistline (which represented the best protected point), and eight other points on the left thigh, leg and foot.

TABLE 1

Skin temperature after walking outdoors in the cold for approximately 15 minutes

		SUBJECT XI*		SUBJECT XIII				SUBJECT XIV				SUBJECT XV			SUBJECT XVI		SUBJECT XVII	SUBJECT XVIII	SUBJECT XIX
Date (1926)		Feb. 16	Mar. 9	Feb. 15	Feb. 17	Feb. 24	Mar. 1	Feb. 11	Feb. 15	Feb. 18	Feb. 25	Feb. 20	Mar. 6	Mar. 13	Mar. 6	Mar. 9	Mar. 9	Mar. 4	Feb. 16
Temperature outdoors, °C.		− 5.0	− 0.5	11.0	1.0	− 0.5	6.0	13.0	12.0	5.5	− 1.0	− 8.0	− 11.0	− 6.0	− 11.0	− 2.4	− 1.0	− 5.0	− 5.0
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Thigh	3	27.3	25.4	26.6	28.6	26.4	26.5	25.8	30.4	26.4	28.3	21.1	22.4	24.7	24.6	26.8	24.5	28.4	29.5
	29	28.9	27.5	25.9	30.0	26.0	27.9	27.1	28.7	24.8	29.1	20.8	23.0	23.2	27.8	32.2	24.8	28.6	30.7
	31	26.5	27.6	26.6	27.6	26.9	27.1	28.3	27.6	27.5	29.1	22.8	24.1	25.7	29.1	34.5	25.5	29.9	31.5
Leg	4	29.6	29.4	31.0	31.8	32.1	31.2	25.9	30.9	28.8	29.0	25.9	27.9	27.6	28.1	26.7	30.2	29.3	29.9
	10	26.9	26.2	28.5	30.9	28.7	28.2	27.0	30.4	27.6	27.6	25.3	26.6	26.2	25.7	23.8	29.5	27.4	29.8
	27	25.4	25.1	27.5	29.4	27.0	27.1	25.9	28.0	26.5	26.5	20.0	22.1	22.5	24.0	26.5	26.3	27.6	28.1
Foot	7	28.1	25.4	32.0	31.7	31.0	29.4	27.2	31.4	30.6	29.7	26.4	20.8	21.4	26.7	26.0	24.2	25.5	31.1
	24	25.2	24.5	30.5	31.4	31.4	28.5	30.4	32.2	32.6	30.3	23.9	19.3	20.0	28.3	25.3	22.9	25.2	30.2
Head	100	23.6	26.5	31.2	30.1	30.9	27.4	28.6	31.2	30.4	31.0	20.7	23.3	22.7	28.8	24.7	22.7	30.9	26.6
	101	26.5	27.6	31.9	30.8	32.1	29.2	30.4	32.4	30.4	29.8	21.5	24.4	23.0	27.9	23.2	22.7	33.8	27.4
	26	30.1	29.1	33.9	35.3	35.9	32.2	32.1	33.9	33.7	33.3	29.7	31.7	30.0	32.3	30.2	28.4	27.2	33.0
Waist, 33 in back		35.0	33.3	33.8	34.6	36.4	32.8	36.6	34.0	34.2	31.4	32.1	33.0	32.8	32.1	35.3	31.5	33.3	37.5
Average		27.7	27.3	30.0	31.0	30.4	29.0	28.8	30.9	29.5	29.6	24.2	24.9	25.0	27.9	27.9	26.1	28.9	30.4

* Eight of the subjects in tables 1 to 7 also served as subjects in a study of the energy requirements of walking, previously reported (Benedict and Parmenter, 1928), and the same subject numbers have been assigned to them in this as in the earlier report.

The results are given in detail in table 1 and, since an equal number of observations at the same points was made on all the subjects, the average value for each person is given at the foot of the table. In practically all instances the average of the points measured was below 30°C., and in one case as low as 24.2°C. The unevenness of skin temperature is strikingly brought out by comparing the high values for the well-protected position 33 with those for the extremities, particularly for positions 7 and 24 on the feet. Indeed, differences of over ten degrees appear in some instances.

One is impressed by the appearance of face temperatures at times extraordinarily high, in spite of the exposure of this part of the body. The loss of heat from the face of an individual, therefore, must be large. This emphasizes again the difficulty of attempting to measure the water loss from the skin in any tests in which the loss from the head, exclusive of the lungs, is not included. The outdoor temperature on a majority of the days was 0°C. or below. Had extremely cold weather been encountered, the temperature differences would have been even greater than noted in table 1.

Skin temperatures before and after a prolonged period of walking, snowshoeing or coasting in cold out-of-doors. The findings on the influence of a relatively short exposure to cold upon skin temperature led to a study of the temperatures before and immediately after prolonged exercise in the cold, such as some of the simpler "winter sports." Nine experiments on five subjects were made, generally in the late afternoon or early evening, and the exercise lasted usually for about one hour. This series (see table 2) gives not simply a general picture of the effect of exposure to cold, but more particularly a comparison of the temperature of the same point before and after exposure. The values obtained in each series have been averaged, unless more than one position in the series was not measured, as was the case with subject XVII.

The average skin temperature[3] prior to exposure was 30°C. or over in all cases. After exposure the average temperature of the same number of points was much lower. The maximum average fall was 7.2°C. with subject XX on February 11, and the minimum was 2.7°C. with subject V on February 13. In practically every case throughout the entire series of measurements there was a fall in temperature of the individual points. Only three increases are noted, and of these two were at the well-protected position 33. The temperature of position 33 was in practically all cases high. Of the exposed positions the temperature of point 26 on the forehead fell noticeably in every instance except one, but in most cases remained much higher than that of any of the protected points save position 33. The cheek temperatures likewise fell and in some instances pronouncedly, a drop of 12° to 13°C. being observed on two different days with subject XVI.

The increased heat production incidental to the physical exercise has resulted in maintaining the normal temperature of the body trunk, but in spite of the protection of winter clothing and in spite of the exercise, the cold has affected the extremities so that the general picture is that of a pronounced drop in the skin temperature. This finding is in accord with the important opinion of Bazett (1927) who states that exposure to cold or to

[3] This term is used for convenience to express the average temperature of the points measured. Obviously the true average of the whole body surface is not known.

TABLE 2

Skin temperatures before and after prolonged walking, snowshoeing or coasting

		SUBJECT V									SUBJECT XIV			SUBJECT XVI						SUBJECT XVII			SUBJECT XX					
Date (1926)		Feb. 11			Feb. 13			Feb. 16			Mar. 11			Feb. 11			Feb. 23			Feb. 26			Feb. 11			Feb. 13		
Temperature outdoors, °C.		− 9.0			− 1.0			− 6.0			− 1.0			− 9.0			− 5.0			+ 2.0			− 9.0			− 1.0		
Activity		Snowshoeing			Coasting and rapid walking			Walking			Walking			Snowshoeing			Rapid walking			Rapid walking			Snowshoeing			Coasting and walking rapidly		
		Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Thigh	3	34.4	32.7	−1.7	32.8	30.8	−2.0	34.6	31.3	−3.3	31.3	25.4	−5.9	32.0	22.6	−9.4	31.5	22.9	−8.6	29.5			35.0	24.2	−10.8	32.8	23 9	−8.9
	29	35.7	30.8	−4.9	33.5	31.6	−1.9	35.2	31.3	−3.9	31.3	26.7	−4.6	32.1	24.2	−7.9	33.3	25.4	−7.9	29.0	21.6	−7.4	34.4	25.8	−8.6	33.2	26.0	−7.2
	31	34.9	28.3	−6.6	32.6	30.9	−1.7	33.8	30.1	−3.7	27.8	24.7	−3.1	32.0	19.8	−12.2	31.0	27.7	−3.3	28.6	22.2	−6.4	32.4	22.7	−9.7	50.1	24.4	−5.7
Leg	4	34.5	34.5	±0.0	31.6	31.6	±0.0	33.3	31.1	−2.2	29.5	25.4	−4.1	31.7	30.9	−0.8	32.1	26.4	−5.7	32.4	29.0	−3.4	34.2	31.5	−2.7	33.5	29.3	−4.2
	10	33.9	33.0	−0.9	32.1	31.0	−1.1	33.9	31.8	−2.1	28.8	23.3	−5.5	31.4	25.9	−5.5	30.7	24.2	−6.5	31.8	28.2	−3.6	32.5	26.1	−6.4	32.8	27.1	−5.7
	27	33.6	29.3	−4.3	30.9	30.2	−0.7	32.8	28.1	−4.7	28.3	21.2	−7.1	31.3	21.2	−10.1	29.4	20.8	−8.6	29.6	23.3	−6.3	32.2	20.5	−11.7	31.2	25.7	−5.5
Foot	7	34.5	29.3	−5.2	34.0	28.5	−5.5	31.9	29.6	−2.3	26.4	20.6	−5.8	32.0	26.2	−5.8	31.1	28.4	−2.7	27.6	26.1	−1.5	36.1	25.3	−10.8	34.0	25.2	−8.8
	24	34.2	26.3	−7.9	34.0	27.0	−7.0	31.9	30.3	−1.6	26.5	20.5	−6.0	31.6	26.7	−4.9	30.0	30.4	+0.4	27.0	24.9	−2.1	35.1	24.1	−11.0	32.8	24.4	−8.4
Head	101	35.2	24.6	−10.6	31.4	25.2	−6.2	32.2	25.4	−6.8	31.1	20.2	−10.9	34.4	22.6	−11.8	33.1	21.4	−11.7	30.0			33.1	29.9	−3.2	31.7	23.4	−8.3
	100	35.0	26.5	−8.5	31.7	26.0	−5.7	30.5	20.6	−9.9	29.6	19.1	−10.5	34.0	21.2	−12.8	34.0	20.2	−13.8	30.4			33.2	31.8	−1.4	32.1	22.8	−9.3
	26	37.6	36.4	−1.2	34.9	34.5	−0.4	36.2	32.6	−3.6	34.3	30.7	−3.6	36.4	35.0	−1.4	35.2	30.4	−4.8	34.5	27.6	−6.9	35.6	32.4	−3.2	35.1	29.5	−5.6
Waist, 33 in back		35.9	37.9	+2.0	36.1	35.6	−0.5	37.1	36.0	−1.1	33.6	35.3	+1.7	32.8	30.7	−2.1	34.2	34.0	−0.2	34.1	30.6	−3.5	34.4			35.7	34.0	−1.7
Average		35.0	30.8	−4.2	33.0	30.2	−2.7	33.6	29.9	−3.8	29.9	24.4	−5.5	32.6	25.6	−7.0	32.1	26.0	−6.1	30.4			34.0	26.8	−7.2	32.9	26.3	−6.6

warmth may cause changes in temperature in one part of the body at the expense of another, without this being demonstrable by measurement of the rectal temperature.

This and the preceding series of observations indicate that the skin temperature, particularly of the extremities and, indeed, of the exposed face, is considerably lowered by exposure to outdoor temperature, even when the exposure is accompanied by the vigorous physical exercise of winter sports. The high temperatures noted on the forehead and cheeks with moderate exposure (see table 1) speak for the large loss in heat that must take place from this area. The irregularity, furthermore, of the face temperatures shows that the face temperature is by no means a function of the time of exposure or of the environmental temperature, but is due to some characteristic peculiar to the individual and cannot be predicted readily.

Influence of exercise (stair-climbing) upon skin temperature. Stair-climbing outdoors. Although from the two series of observations just discussed it is seen that there is a decided fall in skin temperature on exposure to cold, even when the exposure is accompanied by physical exercise, the degree of exercise could not be controlled and the time element after the exercise ceased varied somewhat. In connection with a series of experiments on the metabolism during stair-climbing on a mountain stairway (Benedict and Parmenter, 1928) we measured the skin temperature before and after climbing. Just prior to the stair-climbing a skin temperature survey was made by a trained observer, who measured fourteen points on the body surface. The subjects then climbed the stairway at the rate of about 45 steps per minute and the moment they arrived at the end of the climb (circa 210 steps)[4] the skin temperatures were again measured by a second observer with a duplicate equipment. No especially heavy clothing or outer wraps were worn for this exercise.

Owing to a delay in starting the experiments, most of the subjects expressed themselves as being distinctly cool, if not indeed cold, before they started to walk up the mountain stairway. This was reflected in the skin temperatures, as shown in table 3. Thus, the average skin temperature at the foot of the stairs was in all cases below 30°C., notwithstanding that at least two well-protected points, 52 and 55, were included in this series. However, at the end of the walking, in spite of the large production of heat and the low initial skin temperatures, the skin temperature was *lower* with all six subjects. Furthermore, of the fourteen points studied with each subject, only three at the most in any set showed a rise, all the others showing a distinct fall. The average fall was not far from 1°C.

[4] Since the ordinary staircase in a house has from fifteen to seventeen steps between floors this ascent of 210 steps corresponded to the effort of climbing to the top of a fourteen-storied building, a not inconsiderable task.

TABLE 3

Skin temperatures before and after climbing a mountain stairway (May 7, 1926)

POSITIONS		SUBJECT II			SUBJECT IV			SUBJECT VIII			SUBJECT XIV			SUBJECT XXI			SUBJECT XXII		
		Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Thigh	3	28.2	26.8	−1.4	26.5	25.6	−0.9	29.0	27.6	−1.4	29.2	28.2	−1.0	27.2	26.4	−0.8	30.2	29.6	−0.6
	29	28.9	28.2	−0.7	27.0	26.1	−0.9	29.5	26.8	−2.7	28.2	26.9	−1.3	28.5	28.3	−0.2	30.1	28.7	−1.4
	31	24.1	24.4	+0.3	27.5	27.1	−0.4	27.6	27.5	−0.1	27.1	27.1	±0.0	26.9	27.8	+0.9	27.4	27.6	+0.2
Leg	4	27.5	26.9	−0.6	25.7	25.9	+0.2	24.6	26.4	+1.8	25.0	25.6	+0.6	25.6	26.0	+0.4	27.6	27.1	−0.5
	10	26.9	25.2	−1.7	26.5	24.8	−1.7	26.3	26.5	+0.2	24.3	24.1	−0.2	26.9	26 4	−0.5	26.8	27.5	+0.7
	27	27.7	25.9	−1.8	25.0	25.2	+0.2	24.7	24.2	−0.5	23.9	24.5	+0.6	25.3	24.8	−0.5	26.5	25.4	−1.1
Trunk, front	55	30.3	29.9	−0.4	31.2	31.8	+0.6	32.9	31.9	−1.0	31.9	31.3	−0.6	32.6	28.5	−4.1	33.8	33.9	+0.1
	52	32.0	31.3	−0.7	31.9	29.2	−2.7	32.7	29.9	−2.8	33.3	31.9	−1.4	32.1	31.2	−0.9	34.1	33.7	−0.4
	19	32.0	30.8	−1.2	31.8	31.0	−0.8	31.4	30.7	−0.7	32.8	30.9	−1.9	33.4	32.2	−1.2	34.4	33.6	−0.8
Hand	18	23.3	22.2	−1.1	23.4	21.9	−1.5	21.8	21.1	−0.7	27.3	26.1	−1.2	22.2	21.3	−0.9	23.2	21.6	−1.6
Arm	11	28.1	28.0	−0.1	26.5	25.6	−0.9	25.8	24.4	−1.4	27.2	25.7	−1.5	26.6	24.9	−1.7	29.0	27.9	−1.1
	22	27.5	26.3	−1.2	29.7	27.8	−1.9	32.5	29.3	−3.2	26.3	24.0	−2.3	28.5	27.3	−1.2	30.1	28.3	−1.8
Forehead	26	30.5	28.7	−1.8	31.5	29.4	−2.1	32.4	31.8	−0.6	32.7	30.7	−2.0	31.2	30.3	−0.9	33.6	32.0	−1.6
Cheek	101	24.7	24.1	−0.6	26.9	26.0	−0.9	25.1	24.3	−0.8	26.8	24.1	−2.7	25.9	25.7	−0.2	28.0	26.7	−1.3
Average		28.0	27.1	−0.9	27.9	27.0	−0.9	28.3	27.3	−1.0	28.3	27.2	−1.1	28.1	27.2	−0.9	29.6	28.8	−0.8

This series of observations leads to the conclusion that, in spite of the great increase in metabolism (amounting to over 500 per cent), the skin temperature actually fell rather than rose as a result of this degree of muscular activity. Since it could reasonably be argued, however, that this fall in temperature might be due solely to the prolonged exposure to cold at the foot of the mountain stairway and to the strong air movement up the draughty, though covered stairway, a second series of experiments was made in which the influence specifically of muscular activity upon the skin temperature was studied, the experiments being so designed as to rule out exposure to cold and wind movement other than that caused by the subject's walking.

Running and stair-climbing indoors. The lack of a long flight of stairs indoors on which the subjects could accomplish an amount of muscular work equivalent to that carried out on the Mount Holyoke stairway made it necessary to change entirely the method of performing the activity. Ten subjects therefore exercised indoors in the Cornelia Clapp Laboratory at Mount Holyoke College, by running along a corridor 60 meters long, up four flights of stairs, along another long corridor, down four flights of stairs at the other end of the building, and back to the starting point. The whole run (i.e., making this circuit twice) occupied about four minutes. Thus a study was made under uniform conditions of environmental temperature (about 20°C.) and with no air movement other than that caused by the subject's running. The measurements included the same fourteen points on the body as were measured on the mountain stairway. Essentially the same clothing was worn as in the outdoor mountain experiments, but the subjects certainly were not unduly cool prior to the exercise. This latter point is shown clearly by the average temperature before running (see table 4), which was in practically all cases over 30°C. Averaging of the temperatures after running, in those cases where all fourteen points or all but one were measured, show that there was an average fall in skin temperature of from 0.6° to 1.6°C. with the exception of subject XI, with whom there was an average increase of 0.4°C., due primarily to striking increases in the temperature of the cheek and the hand. Visible perspiration was noted only in the case of subjects I and XXIV. The temperatures of the individual points on the several subjects were almost universally lower after the exercise. The few instances of increased temperature occurred (as with subject XI) for the most part on the hand and cheek.

The general picture, therefore, is that even after engaging in the severe muscular exercise of running through the corridors and up and down stairs for four minutes, during which time the metabolism was certainly increased at least 600 or 700 per cent (which called for an increased heat loss), these young women had on the average a distinctly lower skin temperature,

TABLE 4

Skin temperatures before and after running up and down stairs indoors (May 27, 1926)

POSITIONS		SUBJECT I			SUBJECT II			SUBJECT VIII			SUBJECT XI			SUBJECT XII			SUBJECT XXIII			SUBJECT XXIV			SUBJECT XXV			SUBJECT XXVI			SUBJECT XXVII		
		Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Thigh	3	32.7	30.5	−2.2	32.0	30.6	−1.4	34.5	32.5	−2.0	28.1	28.6	+0.5	31.4	30.3	−1.1	29.9			31.6	29.7	−1.9	31.4	30.3	−1.1	33.3	32.6	−0.7	32.6	29.3	−3.3
	29	33.5	31.1	−2.4	32.4	31.0	−1.4	32.3	31.6	−0.7	30.4	29.1	−1.3	31.5	30.1	−1.4	30.8	30.2	−0.6	32.2	29.9	−2.3	31.6	29.8	−1.8	32.6	31.6	−1.0	30.9	28.4	−2.5
	31	31.2	29.4	−1.8	29.0	28.6	−0.4	31.5	30.4	−1.1	31.5	30.8	−0.7	30.5	30.0	−0.5	28.3	28.1	−0.2	31.2	29.1	−2.1	32.9	31.9	−1.0	31.6	31.1	−0.5	28.7	27.8	−0.9
Leg	4	28.9	28.8	−0.1	31.3	28.9	−2.4	30.8	30.7	−0.1	29.0	29.4	+0.4	30.4	30.2	−0.2	31.4	30.6	−0.8	30.0	28.9	−1.1	30.1	29.2	−0.9	30.4	30.6	+0.2	30.4	30.1	−0.3
	10	29.4	27.6	−1.8	30.9	29.6	−1.3	30.5	30.1	−0.4	27.6	28.2	+0.6	29.5	29.4	−0.1	29.9	29.3	−0.6	29.3	30.1	+0.8	28.1	28.2	+0.1	32.4	32.2	−0.2	29.9	29.0	−0.9
	27	28.0	26.2	−1.8	30.7	29.4	−1.3	30.0	28.4	−1.6	28.0	26.4	−1.6	27.8	27.0	−0.8	28.4	27.7	−0.7	28.8	28.5	−0.3	28.2	26.9	−1.3	32.2	29.8	−2.4	30.1	27.2	−2.9
Trunk, front	55	34.3	32.5	−1.8				34.5	32.5	−2.0	33.0	31.3	−1.7	33.1	32.6	−0.5	32.7	32.2	−0.5	34.4	32.8	−1.6	33.3	32.3	−1.0	34.7	34.2	−0.5	33.6	30.7	−2.9
	52	32.7	31.6	−1.1	35.4	32.5	−2.9	34.5	33.9	−0.6	33.7	33.7	±0.0	34.4	33.6	−0.8	34.8	33.8	−1.0	34.5	32.8	−1.7	34.7	33.7	−1.0	35.1	34.6	−0.5	35.2	32.3	−2.9
	19	33.4	31.2	−2.2	34.6	33.0	−1.6	32.6	31.0	−1.6	32.8	32.1	−0.7	33.6	33.1	−0.5	34.7	33.8	−0.9	34.0	33.0	−1.0	33.5	32.6	−0.9	34.9	33.3	−1.6	33.3	31.0	−2.3
Hand	18	32.0	31.8	−0.2	30.8	28.1	−2.7	32.7	32.8	+0.1	26.3	29.6	+3.3	30.0	29.0	−1.0	26.5	25.4	−1.1	29.1	30.3	+1.2	28.1	29.7	+1.6	29.5	26.8	−2.7	27.0	24.0	−3.0
Arm	11	33.0	31.0	−2.0	30.9	31.0	+0.1	32.9	31.3	−1.6	29.8	29.8	±0.0	32.6	30.9	−1.7	31.1	29.8	−1.3	32.4	31.5	−0.9	30.1	29.3	−0.8	32.7	31.4	−1.3	31.6	30.9	−0.7
	22	33.5	32.6	−0.9	31.0	30.2	−0.8	32.8	31.0	−1.8	29.1	30.1	+1.0	33.6	32.7	−0.9	30.1	28.5	−1.6	30.7	29.1	−1.6	30.8	30.1	−0.7	31.8	32.9	+1.1	30.0	29.8	−0.2
Forehead	26	33.5	33.9	+0.4	33.7	32.3	−1.4	34.8	33.8	−1.0	32.7	34.2	+1.5	34.3	33.7	−0.6	34.0	33.4	−0.6	34.0	32.9	−1.1	33.9	33.6	−0.3	33.8	34.1	+0.3	33.4	33.9	+0.5
Cheek	101	34.1	35.5	+1.4				35.5	36.2	+0.7	30.8	35.5	+4.7	33.5	34.1	+0.6	29.9	29.2	−0.7	33.0	33.8	+0.8	33.5	34.1	+0.6	31.3			29.8	29.8	±0.0
Average		32.2	31.0	−1.2				32.8	31.8	−1.0	30.2	30.6	+0.4	31.9	31.2	−0.7	30.9	30.2	−0.8	31.8	30.9	−0.9	31.4	30.8	−0.6	32.6	31.9	−0.7	31.2	29.6	−1.6

which it is difficult to ascribe solely to the effect of the wind movement created by the subjects in running.

Detailed study of skin temperature before and after exercise indoors—Subject XI. Because of the marked rise in temperature noted on the face and hands of subject XI after exercise indoors (see table 4), two further experiments were made with this subject; 1, to determine whether with her exercise had an effect different from that noted with the other young women, and 2, to determine what would be the effect of repeated, short periods of exercise on the skin temperature. In the first experiment subject XI ran in Clapp Laboratory twice over the same course as in the previous series, exercising for four minutes Skin temperature measurements on the same fourteen points studied in the earlier experiment with her (see table 4) were made before and immediately after this exercise and, in order to study the return of the skin temperature to normal conditions, these measurements were repeated three times thereafter at approximately 15-minute intervals. During all the measurements, both before and after the exercise, the subject remained quiet. When it appeared that the temperature in the periods of repose had reached the initial level, a second experiment was made, in which the effect of repeated exercise was observed. In this experiment the subject ran over the same course once, performing but one-half the original exercise and, since it was desired to shorten as much as possible the interval between the runs, the temperatures of seven only of the fourteen points were determined immediately afterwards. A second and a third time she repeated the single run, and the same seven points were measured. The results of both experiments are given in table 5.

In the first experiment the average temperature of the 14 points prior to exercise was 32.5°C. After 4 minutes of exercise there was a consistent fall in temperature at all points except positions 52 and 101, where increases of 0.6° and 1.0°C. were observed, confirming in a way the previous finding in the first series (table 4), where position 101 showed a rise of 4.7°C. In general, however, there was a distinct fall in temperature, that is, from 32.5°C. before the exercise to 31.7°C. afterwards. The subsequent course of the temperature was a slow rise, which ultimately resulted, forty minutes after the exercise ceased, in an average of 32.8°C.; in other words, a return to a value slightly above the initial average.

In the second experiment the temperatures were determined only at seven points. The averages for these may not be compared directly with the averages for the fourteen points measured in the first experiment, and we have therefore included in table 5, for comparison purposes, the averages in the first experiment for the same seven points as were measured in the second experiment. In experiment II, even when the exercise consisted of running over the course but once, the average temperature of the seven points measured (32.1°C.) was practically the same as that of the

same seven points measured in period 1 of experiment I and distinctly lower (1.1°C.) than that in period 4 of experiment I. Furthermore, every point studied in experiment II showed a fall in temperature after the subject had run once. The seven points selected fortunately were sufficiently varied in nature to give almost identically the same general picture of a fall in skin temperature following exercise as was noted in the first experiment.

TABLE 5

Detailed study of skin temperature before and after exercise indoors—Subject XI (June 2, 1926—Room temperature, 23°C.)

POSITION		EXPERIMENT I					EXPERIMENT II		
		Before run (2.06 p.m.)	After 4-minute run (2.10–2.14 p.m.)				Immediately after running		
			Period 1 (2.14 p.m.)	Period 2 (2.30 p.m.)	Period 3 (2.34 p.m.)	Period 4 (2.56 p.m.)	First run (3.03 p.m.)	Second run (3.10 p.m.)	Third run (3.14 p.m.)
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Thigh	3	31.8	29.9	31.2	31.6	32.5	31.0	30.4	30.1
	29	30.9	30.6	31.3	31.9	32.3			
	31	30.3	29.9	30.1	30.2	30.1			
Leg	4	31.2	29.6	30.5	30.7	31.8			
	10	30.2	28.8	29.8	30.1	30.1	29.4	28.8	28.5
	27	29.6	29.0	30.0	30.1	29.8			
Trunk, front	55	32.9	31.8	32.2	32.4	33.0	32.2	31.6	31.0
	52	35.0	35.6	33.5	34.6	35.2			
	19	34.5	32.7	33.0	33.2	34.2			
Hand	18	33.9	32.7	34.1	33.9	33.8	32.3	31.9	32.0
Arm	11	32.6	30.8	31.8	32.4	33.0	32.1	31.4	30.5
	22	33.0	32.9	32.6	33.2	33.1			
Forehead	26	34.1	34.0	32.9	33.8	34.6	32.9	32.2	34.3
Cheek	101	35.2	36.2	35.5	35.5	35.5	34.7	36.0	36.5
Average of 14 points		32.5	31.7	32.0	32.4	32.8			
Average of 7 points		33.0	32.0	32.5	32.8	33.2	32.1	31.8	31.8

After the second run in experiment II there was a further fall in temperature of all points save on the cheek. The considerable rise in the temperature of the exposed cheek resulted in an average fall for the entire seven points of but 0.3°C. After the third run the temperature of the four covered points, namely, positions 3, 10, 55, and 11, fell noticeably still further. On the other hand, the temperature of the hand and the face, particularly the forehead, showed a distinct recovery.

Further consideration of these data shows that the magnitude of the fall in skin temperature following exercise is not appreciably different at various parts of the body, even if there is a difference in the amount of wind movement about these parts or even if some parts are covered and others unprotected. Thus, position 55 and the three points measured on the leg were all covered by clothing, but there was probably more wind movement about the leg than about the abdomen. Conversely, there was wind movement both about the arm and about the leg, but the leg was covered and the arm was not. In spite of these differences, the fall in skin temperature after exercise was practically the same at all points.

Following the two experiments reported in table 5, subject XI stood for about an hour, and ten consecutive series of observations at gradually lengthened intervals were made at all fourteen points, to see when the temperatures would return to normal as a result of quiet standing. Curves based upon these observations show that as a result of three periods of running there was a rapid fall in the average skin temperature, and that with rest there was an equally rapid rise, the temperature finally reaching and remaining essentially that observed before the exercise.

On closer study of subject XI, therefore, no abnormal picture is obtained. The exercise resulted in a distinct lowering of skin temperature on the average, even when the exercise was repeated, and following rest the skin temperature rapidly approached its normal level.

Effect of wind movement on skin temperature. Although a noticeable fall in skin temperature occurred with these young women as a result of considerable muscular exercise, it was felt that the wind movement produced by the running of the subject might of itself account for a part, at least, of the lowered skin temperature. The influence of wind movement alone upon the skin temperature, under clothing, of a young woman (subject XXVIII) remaining perfectly quiet was, therefore, next studied at the Nutrition Laboratory. Thus there would be no increase in metabolism as a result of activity which would raise the temperature of the body and counteract any fall in skin temperature caused by wind movement, and hence any fall in skin temperature would be ascribable solely to wind movement. It has been computed that in the stair-climbing experiments our subjects ran on the average at the rate of four miles per hour, explained by the fact that they could not ascend the stairs at the same speed at which they ran on the level.[5] In the experiments made at the Nutrition Laboratory, subject XXVIII stood fully clothed, with a silk dress on, facing the blast from an electric fan placed directly in front of her, seven feet away.

[5] The arm and particularly the leg motions were irregular, with a period of repose compensated by rapid movement as the limbs moved forward to take a new step. The average speed was in all cases that of the average body movement in forward progression.

The wind velocity was calculated from an anemometer as being 200 meters per minute, i.e., six and one-half miles per hour, certainly somewhat in excess of the rate of wind movement produced by our subjects when running in the corridors on the level.

TABLE 6

Effect of wind movement on skin temperature—Subject XXVIII (June 22, 1926)

POSITION		EXPERIMENT I			POSITION		EXPERIMENT II			
							In still air		After 5 minutes in wind	
		In still air (11.20 a.m.)	After 5 minutes in wind	Difference			4.46 p.m.	4.54 p.m.	5.07 p.m.	5.14 p.m.
		°C.	°C.	°C.			°C.	°C.	°C.	°C.
Head	26	33.8	33.2	−0.6	Head	26	33.9	34.7	33.7	34.5
	100	32.9	31.8	−1.1		101	34.0	34.2	32.9	33.7
	101	33.0	31.6	−1.4						
Trunk	51	32.8	32.9	+0.1		52	35.1	35.2	34.6	35.0
	52	34.4	33.3	−1.1		53	35.0	35.2	35.1	35.0
	53	35.1	34.6	−0.5		54	35.5	35.4	34.9	34.8
	54	35.1	33.6	−1.5	Trunk	55	34.6	34.2	33.8	33.8
	55	33.9	32.8	−1.1		15	35.0	34.8	34.7	34.7
	19a	33.6	31.9	−1.7		13	35.4	35.4	35.3	35.2
	19	34.0	32.8	−1.2		35	33.4	33.3	33.2	33.4
	14	34.6	34.1	−0.5		33	33.5	33.9	34.4	34.4
	33	33.5	34.8	+1.3	Arm and hand	22	30.4	31.1	30.7	31.1
Arm and hand	22	30.5	30.1	−0.4		11	31.3	32.3	30.5	31.5
	11	31.5	29.8	−1.7		18	31.9	32.6	31.5	32.5
	18	31.9	31.4	−0.5						
Thigh and leg	31	31.2	30.1	−1.1		31	31.3	31.1	30.9	30.8
	3	32.6	31.7	−0.9		3	33.1	33.4	32.7	32.6
	29	33.0	32.2	−0.8	Thigh and leg	29	33.1	32.7	32.8	32.8
	27	30.0	29.6	−0.4		27	30.3	30.5	29.8	30.1
	10	30.6	29.7	−0.9		10	30.5	31.4	30.6	30.9
	4	31.8	31.2	−0.6		4	31.4	32.2	31.6	31.8
Average		32.8	32.1	−0.7	Average		33.1	33.3	32.8	33.1

Before the fan was set in motion, the skin temperature was measured at 21 points on the body (see table 6) and was found to average 32.8°C. The subject then stood for five minutes in the blast of air, the room temperature during this time being 22.7°C. (dry bulb) and 18.6°C. (wet bulb). As a result the average skin temperature fell to 32.1°C., i.e., an average fall of 0.7°C.

In a second experiment with this same subject the fan was directed similarly against the body of the subject while standing, but was moved up and down in a vertical direction, so that the air played over the entire body surface from head to foot. In still air two consecutive series of observations on 19 points showed an average skin temperature of 33.1°C. at 4:46 p.m. and 33.3°C. at 4:54 p.m. The wind was then directed against the body for five minutes in a vertical direction, and at 5:07 p.m. the average temperature was 32.8°C., i.e., there was an average fall of 0.5°C. This fall of 0.5°C. is comparable to the fall of 0.7°C. noted in the first fan experiment with subject XXVIII. The two experiments may, therefore, be looked upon as duplicates and the conclusion is that the vertical movement of the fan had the same effect as the wind directed at one part of the body. After seven minutes of repose in still air the average temperature had again risen to 33.1°C.

In the experiments reported in table 4 the skin temperature was lowered on the average 1° C. by running up and down stairs. Comparison with the data given in table 6 indicates that a forced wind blast, admittedly higher in velocity than could possibly have been produced by the average running speed, resulted in a fall in skin temperature on the average somewhat less than the average fall in those instances where muscular activity as well as wind movement occurred, i.e., the running experiments. The picture is much the same, whether all the values in table 6 are averaged in each series or only the fourteen points comparable to those listed in table 4. Although in general there is a marked uniformity in the reaction to wind and activity of the skin temperature at different parts of the body, whether protected or exposed, the data given in the various tables throughout this article show that the distinctly exposed parts of the body, especially the cheek and the forehead, may have a distinctly different temperature reaction from that of the protected parts, and hence sharp comparisons of the results in the different series of experiments are difficult. Yet the data as a whole suggest that muscular activity plays an individual rôle in lowering the skin temperature, independent of wind movement or of exposure to cold.

From subsequent measurements of the respiratory exchange we are convinced that the heat production of these girls while running was on the average not far from 6 calories per minute or 30 calories for five minutes. If we assume that the energy expenditure during quiet standing was 6 calories in five minutes, the excess heat production due to running was 24 calories. The average body weight of these girls was 60 kilograms. Since the specific heat of the body is about 0.83, the hydro-thermal equivalent would be 50 kilos. To store the excess 24 calories would, therefore, raise the temperature of the entire body about 0.5°C. Rectal temperatures were not taken, but it is known that severe muscular work raises the

rectal temperature. It is a significant fact, therefore, that the peripheral temperature actually fell rather than rose during the running experiments, and that only a part of this fall might be attributed to the effect of the rather moderate wind movement to which the young women were subjected while running.

Effect of walking on a treadmill, without wind movement, on skin temperature. In order to study the effect of muscular effort without wind movement, experiments were made upon a rolling platform or treadmill at the Nutrition Laboratory. This could be inclined at any desired angle and the rate of movement adjusted so that in a given length of time the actual amount of work involved in either the mountain climbing experiments or the running experiments along the corridors and on the stairs in Clapp Laboratory could be approximated. Under these conditions the subject would be walking in a room of constant temperature (20°C.), and, since she would remain in an essentially fixed position on the treadmill with the arms held in place on two upright supports, there would be no wind movement except that caused intermittently by the changes in position of the legs, which might affect the leg temperature.

Subject XXVIII again volunteered for this experiment. Prior to the walking a general survey of the skin temperature was made at 21 points, and the average temperature was found to be 33.3°C. at 12:25 p.m. The treadmill was then placed at such an angle that the subject walked up a grade of about 17 per cent at a rate of 55 meters per minute (i.e., 2 miles per hour) for a period of five minutes. At the end of this exercise, which would usually cause a rise of at least 500 to 600 per cent in the metabolism, the average skin temperature had fallen to 32.5°C., only one point (52) showing a slight, probably insignificant, rise. About five minutes later, during which time the subject remained standing, there was an almost immediate return to the average value noted prior to the exercise, seventeen of the 21 points showing a positive increase and the average temperature being 33.1° and 33.4°C in two successive series. The room temperature during this experiment was 23°C. (dry bulb) and 19°C. (wet bulb).

The results obtained in this experiment with subject XXVIII led to a further study along this same line with two other women at the Nutrition Laboratory. These subjects (XXIX and XXX) entered the large laboratory where the treadmill was located in the morning, and remained in this environment throughout the entire series of experiments. The temperature of the dry bulb was 20.8°C. and the wet bulb 13.7°C. at 9:30 a.m. Three series of observations on 21 positions on subject XXIX at 9:26, 9:41 and 9:55 a.m. showed an average skin temperature of 32.2°C. in each case, thus indicating constancy. At 10:03 a.m. she walked on the inclined treadmill for five minutes. In a series of observations made immediately after this exercise (at 10:09 a.m.) the skin temperature aver-

aged 31.8°C., that is, there was again the characteristic, though small, fall in temperature found with walking. Of the 21 points measured seventeen showed a lower temperature after walking, two no change, and two (positions 4 and 33) an increase of 0.4° and 0.3°C., respectively. In a subsequent series at 10:19 a.m. the average temperature was 32.2°C., and at 10:29 a.m. it was 32.4°C., all of the individual points having temperatures essentially the same as prior to the exercise.

The second subject (XXX) had been in the laboratory for one hour before observations were made on her. At the beginning of her experiments the dry bulb was 21.2°C. and the wet bulb 14.1°C. Two series of measurements on 22 points showed a constant average temperature of 32.6°C., both at 10:35 and 10:45 a.m. She then walked on the inclined treadmill for five minutes. Immediately after the treadmill was stopped (10:57 a.m.) a third series of measurements began, which showed an average temperature of 32.2°C., or the characteristic drop in temperature noted with all the other subjects. At 11:06 a.m. the skin temperature had risen again to an average of 32.6°C., and at 11:16 a.m. it was also 32.6°C., i.e., it had attained constancy.

These treadmill experiments with these three subjects confirm each other fully, and prove that even when the effects of wind movement and exposure to cold are seemingly for the greater part ruled out, there is a *distinct drop in the average peripheral temperature as a result of muscular activity for five minutes, in spite of the fact that the metabolism is greatly increased and the heart rate is somewhat increased and hence a greater heat loss would be expected.* In this connection it is interesting to note that as early as 1876 Adae, using a mercury thermometer, recorded a decrease in the temperature of the closed hand as a result of walking, and found that only when the exercise was continued for a long time was there finally a slight increase in temperature.

Effect of exercise, when nude, upon skin temperature. Although the elements of wind movement and of exposure to cold were minimized by having these subjects walk on a treadmill in a laboratory at essentially a constant temperature, it is conceivable that as a result of the muscular movement there may have been an interplay in the layers of air under the clothing resulting in an unusual ventilation of the air spaces next to the warm flesh under the clothing, i.e., a sort of "bellows" effect of the clothing. The question therefore arose as to what effect exercise would have upon the skin temperature when the subject was nude.

Fortunately one of our subjects (XXIX) was an artist's model, who had been accustomed for many years to posing nude and who had been for years a subject for experiments in the Nutrition Laboratory. She disrobed at 10:38 a.m. (following the treadmill experiment with her when clothed) and remained nude in the laboratory until 11:35 a.m., when the

first series of skin temperature measurements (representing 22 points) was made with her (see table 7). This series showed an average skin temperature of 31.0°C. At this time the dry bulb temperature in the room was 21.7°C. and the wet bulb was 14.4°C. A second series at 11:44 a.m. showed an average temperature of 30.8°C., a third series at 11:51 a.m.

TABLE 7

Skin temperature of subject XXIX, nude, before and after walking on treadmill

POSITION		BEFORE EXERCISE				AFTER EXERCISE					
		(a) 11.35 a.m.	(b) 11.44 a.m.	(c) 11.51 a.m.	(d) 11.59 a.m.	(e) 12.11 p.m.	(f) Diff. (e>d)	12.20 p.m.	12.30 p.m.	12.40 p.m.	12.54 p.m.
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Head	26	34.0	34.0	34.0	34.0	33.6	−0.4	33.8	34.4	33.6	34.0
	101	31.2	31.2	31.2	31.0	30.9	−0.1	31.0	31.1	30.9	31.0
	100	30.6	30.8	30.8	30.7	30.5	−0.2	30.5	30.7	30.6	30.7
Trunk	19	33.0	33.0	32.9	33.0	32.7	−0.3	32.8	32.9	32.6	32.6
	19a	32.4	32.5	32.2	32.5	32.4	−0.1	32.6	32.6	32.4	32.4
	51	32.8	32.5	32.4	32.4	32.4	±0.0	32.2	32.7	32.2	32.5
	52	32.0	31.7	31.9	32.1	32.1	±0.0	32.1	32.1	31.9	32.1
	53	32.6	32.5	32.3	32.7	32.4	−0.3	32.8	32.4	32.3	32.4
	54	31.1	31.1	30.8	30.6	30.6	±0.0	30.7	30.9	30.7	30.7
	55	29.8	29.9	29.6	29.4	29.2	−0.2	29.1	29.3	29.2	29.2
	14	33.3	33.1	33.1	33.1	32.9	−0.2	33.1	33.2	33.1	33.2
	33	30.5	30.5	30.8	30.7	30.8	+0.1	31.2	31.0	30.7	30.7
Hands	18	31.6	30.0	30.2	30.1	29.9	−0.2	29.5	29.2	29.3	28 9
	18a	31.5	30.4	30.3	30.1	30.1	±0.0	29.1	28.6	28.8	29.0
Arm	11	31.6	31.1	31.1	29.8	30.1	+0.3	30.1	29.9	29.5	29.3
	22	28.8	29.2	29.2	28.9	28.9	±0.0	29.0	29.2	29.1	29.1
Thigh and leg	31	27.6	27.5	27.5	27.5	27.2	−0.3	27.3	27.3	27.3	27.3
	29	29.3	29.2	29.1	29.1	28.6	−0.5	29.0	29.2	29.1	29.2
	27	28.4	28.3	28.3	28.2	27.9	−0.3	28.9	29.1	28.8	28.6
	3	29.4	29.1	28.3	28.7	28.4	−0.3	28.4	28.6	28.7	28.8
	4	30.9	31.1	31.2	31.2	31.8	+0.6	31.8	31.5	31.5	31.5
	10	29.3	29.3	29.5	29.9	30.0	+0.1	30.4	30.7	30.5	30.1
Average		31.0	30.8	30.8	30.7	30.6	−0.1	30.7	30.8	30.6	30.6

an average of 30.8°C., and a fourth series at 11:59 a.m. 30.7°C. This finding confirms many earlier experiments with her, in which after disrobing at an environmental temperature of 21°C. her skin temperature has reached constancy in essentially an hour and a half, i.e., the same length of exposure as that to which she was subjected in the experiment under discussion. At 12:05 p.m. she walked on the treadmill for five

minutes at the same speed and under exactly the same conditions as formerly. The skin temperature then averaged 30.6°C., which represented a slight, but probably insignificant fall of 0.1°C., explained by the fact that although there was a fall at 13 points, there was no change at 5 points, and a rise at 4 points. The subject remained standing in the laboratory, and four series of readings at 10-minute intervals gave average temperatures of 30.7°, 30.8°, 30.6° and 30.6°C. The initial average skin temperature in this series of 30.7°C. after an hour's cooling is, to be sure, two degrees below the average temperature of the normally clothed woman reported in table 6, but nevertheless is relatively high in comparison with the environmental temperature and presumably indicates a moderate blood flow.

With this particular subject we had to deal not with wind movement (other than that of intermittent leg motion), not with a falling skin temperature due to sudden exposure to cold (for the subject had been nude long enough for the skin temperature to reach constancy), and not with any possible disturbance in the layer of air next to the skin under the clothing, but with the effect of exercise *per se*. Although the *average* skin temperature did not fall appreciably under these conditions, there was an actual lowering at 13 of the 22 points measured, no change at 5 points, and a rise at but 4, in spite of an increased metabolism of over 600 per cent during a period of five minutes.

CONCLUSIONS

Skin temperature measurements have thus far been but little utilized in physiological or pathological studies, and this field of research is relatively untilled. Hence it is not surprising that the general picture presented by our data is not clearer. With the newly awakened interest in the significance of the skin temperature of humans, however, there is bound to be much discussion of the rôle played by this factor, and in so far as possible normal values should be available. We have therefore considered it justifiable at least to put our results on record as facts definitely established by an accurate technique, which should have a specific value as normal data, whatever may be their ultimate interpretation. We cannot hope to discuss these findings adequately at this stage in the development of our knowledge of skin temperature, and our conclusions are based simply upon the information we have thus far secured, in full realization that further investigations are needed to clarify and strengthen them.

Since the heat loss is almost directly proportional to the difference between the skin temperature and the temperature of the environment, measurements of skin temperature should be helpful in indicating the rate of heat loss from different parts of the body, especially in any study of the loss of heat by radiation. About seven years ago, in an unpublished

research carried out in the Nutrition Laboratory in collaboration with Dr. C. G. Abbott, now Secretary of the Smithsonian Institution, it was found by means of his pyranometer that the human skin acts essentially as a black body. In 1924 Cobet and Bramigk state that Rubner reported to them in a personal communication that he had unpublished experiments which likewise showed that the human skin acts as a black body, and Cobet and Bramigk report that their own findings confirm this completely. Thus it is clear that the skin temperature, as such, may be an excellent index of the amount of heat lost by radiation.

The facts with regard to the variations in skin temperature, as here recorded, are, we believe, definitely established. Thus, exposure to severe cold, with or without strenuous muscular exercise, results in a lowering of the skin temperature, chiefly of the extremities, a lowering which cannot be counteracted even by the large production of heat in the strenuous exercise of winter sports. During short periods of severe exercise, such as walking indoors on a treadmill or walking upstairs, the metabolism may be increased more than five or six hundred per cent, and yet the peripheral temperature will *simultaneously be lowered* temporarily. Even when the skin has been previously cooled by removal of clothing and there has presumably been some restriction of cutaneous circulation, moderately severe muscular exercise (for 5 minutes) may raise the metabolism five or six hundred per cent without causing an increase in skin temperature, as would be expected when provision must be made for increased heat loss.

Such phenomena may frequently be expected in this new line of study. As our research progressed and the experimental data accumulated, we tried to alter our experimental conditions so that the influence of wind movement and of changes in the vaporization of water from the skin might be eliminated, in so far as possible. Since we have taken these factors into consideration, it seems to us that the most plausible explanation of these alterations in skin temperature following exercise is that we have here to deal possibly with a vaso-constriction of the peripheral vessels, resulting in a temporary transport of blood from the periphery to the muscles, at least in the initial stages of muscular work. This explanation we put forth, however, only as an hypothesis, and we realize that much more experimental data are needed to clarify this problem.

At this point we wish to express to Prof. H. C. Bazett of the University of Pennsylvania and to Prof. A. H. Turner of Mount Holyoke College our deep appreciation of their friendly criticisms, which have been greatly stimulating to us. Material changes in plans and in residence make it impossible for us to continue this research in a coöperative manner. It is hoped that the Nutrition Laboratory will subsequently be able to make further experiments dealing with skin temperature and muscular work and particularly that other laboratories will include this problem in their programs.

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THE CORRELATIONS BETWEEN VITAL CAPACITY AND VARIOUS PHYSICAL MEASUREMENTS IN ONE HUNDRED HEALTHY MALE UNIVERSITY STUDENTS[1]

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Received for publication September 10, 1928

The relationship between pulmonary capacity and other physical measurements is a problem of both theoretical and practical interest. As was emphasized by Shepard and Myers (1925), there is still much disagreement as to the best standard for determining the normal vital capacity of a given individual. The interpretation of individual spirometric observations is limited through lack of knowledge as to the normal variation in vital capacity and as to the correlation between vital capacity and other measurements of the body. Myers (1925) has reviewed the literature and has tabulated norms for vital capacity according to sex, stature, sitting height, chest girth, body weight and surface area. Lundsgaard and associates (1918, 1923) introduced the "chest volume" as an index of lung capacity. Wittig and Polczak (1926) advocate the breath-holding test for this purpose. Jackson (1927) has recently shown that the correlation between vital capacity and chest expansion is relatively low. The present study was undertaken to determine, in a selected group of healthy young men, the exact correlation between vital capacity and various physical measurements, including both those previously used and a number of new measurements and modifications which might yield a better normal standard.

METHODS OF MEASUREMENT. The young men used in the present study were university students (chiefly freshmen and sophomores) taken from the regular gymnasium classes. All cases with history of pulmonary or cardiac disorders which might affect the vital capacity were excluded; otherwise the selection was at random. The observations were made during the spring of 1927, at average room temperature of 77°F. (range 72 to 82°F.).

The same procedure was used in each case as follows. All clothing was removed. The name, age (at last birthday), time and character (full or light) of last meal, and body weight (to quarter pound, on standard Fairbanks scale) were recorded. The subject then rested (supine) for a

[1] This study was made with the aid of a grant from the Medical Research Fund of the University of Minnesota.

period of five minutes or more. While still recumbent, the pulse rate was counted for one minute using a stop watch. Without the knowledge of the subject, the examiner next counted the respiratory rate for one minute, and also noted the type of quiet respiration (abdominal, costal or mixed).

The standing height was measured with the sliding rod on the Fairbanks scale (to the quarter inch; later transferred to metric system). Next the sitting height was measured with the subject sitting on a table, knees extended, with back to the wall, on which was placed a vertical metric scale in millimeters. In 69 cases the sitting height was also taken with knees flexed, according to Dreyer's method. The vital capacity was then carefully measured with a Sanborn water spirometer, three trials being made, with subject standing. The highest reading was taken as the vital capacity.

The thoracic measurements were likewise taken standing, using a Martin's pelvimeter for the diameters and a linen or metal metric tape (in millimeters) for the girths. All the measurements (except sternal length) were taken at 1, end of quiet respiration (expiration); 2, at maximum inspiration, and 3, maximum expiration. The thoracic diameters were taken according to Lundsgaard, including 1, sternal length, suprasternal notch to sterno-ensiform junction; 2, midsagittal, at level of third chondrosternal junction; 3, transverse, on sixth rib in the midaxillary region. The trunk girths were measured, at each of the three respiratory stages, at three levels: 1, thoracic at nipple level; 2, abdominal at umbilical level; 3, epigastric, measured half way between the other two levels.

After a short period of rest (recumbent), the breath-holding tests were made, with subject standing, mouth and nares firmly closed. No swallowing movements or previous deep breathing were permitted. Using a stop watch, the maximum period during which the breath could be held voluntarily was recorded 1, at the end of quiet respiration; 2, after a single maximum inspiration; and 3, after a maximum expiration. In some doubtful cases the test was repeated after a period of rest. In general, excellent coöperation was given by the subjects, who exerted themselves fully in the various tests. We are greatly indebted to Prof. F. W. Luehring and various instructors in the department of physical education; likewise especially to Mr. Wayne C. Rydburg for assistance in making the examinations.

The various statistical constants and their probable errors (table 1) and also the correlations with vital capacity (table 2) were calculated, using the formulas according to Pearl (1923). The probable errors make it possible to estimate the significance of the various measurements and their differences when compared, especially the differences in the degrees of correlation with vital capacity. The regression lines were constructed in all cases. Since the regressions are not always strictly linear (in which case the coefficient of correlation, r, is not accurate), the correlation

ratio (η) has also been calculated in each case. In interpreting the correlation ratio the correction for the number of arrays must be considered, according to the formula $\sqrt{\frac{K-1}{N}}$. Since the number of arrays (K) for the vital capacity is 16, and the number (N) is always 100, the corresponding correction factor is 0.386; so that in general where the correlation ratio is less than 0.386 no significant association can be inferred. The frequency distributions are omitted for lack of space, but in general they appear fairly normal in character.

OBSERVATIONS AND RESULTS. *Vital capacity.* The mean vital capacity (table 1) for the present group is 4406 cc., which is slightly above the general student average (4383 cc.) found by Jackson (1927). The coefficient of variation in the present group, 13.05 per cent, is slightly below that previously found for the general student average (14.54). It may be noted that in general the vital capacity is more variable than stature, and slightly more variable than body weight; but it is slightly less variable than the pulse rate, and much less variable than the respiratory rate or the chest expansion (see table 1).

Age. The age of the present group ranges from 17 to 22 years, with a mean of 19.4 years (table 1). On account of this small range, the coefficient of variation for age (6.24) is rather low. The coefficient of correlation between age and vital capacity (0.041 $\pm$0.067) is insignificant, as might be expected in this group.

Stature. The stature or standing height ranges from 157 to 187 cm., with mean of 173.08 cm. (table 1). This is slightly below the general student average (174.46). The coefficient of variation (3.48) is also slightly below the general student average (3.66). The coefficient of correlation between stature and vital capacity is 0.666 $\pm$0.038. This is slightly (but insignificantly) higher than the corresponding correlation (0.568 $\pm$0.013) found by Cripps, Greenwood and Newbold (1923) in 1238 Air Force candidates. As shown in table 2, there is in the present group no significant correlation between stature and age, a remarkable finding which was noted also by Jackson (1927) in the larger student group.

Sitting height. The sitting height (table 1) averages 86.97 cm., measured with knees extended. Measurements according to the technique of Dreyer (1919, 1921) (with knees flexed) for "stem length" gave nearly the same mean, although varying in individual cases. The coefficient of correlation between sitting height and vital capacity (table 2) is 0.574 $\pm$0.045, which is insignificantly above the corresponding correlation (0.527$\pm$0.014) found by Cripps, Greenwood and Newbold (1923) in their larger group. The correlation between the vital capacity and the square of the sitting height (used by Dreyer) is 0.579 $\pm$0.045, or practically the same as that for the sitting height. The results of the present study, like those of other recent

observers, fail to confirm Dreyer's conclusion that the sitting height (or "stem length") is less variable than the stature and more closely correlated with vital capacity.

Body weight. The mean body weight of the present group (table 1), 62.03 kilograms, like the stature is slightly below the general student average (64.157). The coefficient of variation (9.37) is also somewhat below the general average (12.27). The coefficient of correlation between body weight and vital capacity in the present group is remarkably high (0.743) in comparison with that found by Cripps, Greenwood and Newbold (1923) for their larger group (0.483). Their correlation between stature and body weight (0.547), however, is closer to that for the present group (0.587).

Pulse rate. The mean pulse rate of the present group (table 1), 78.61 per minute, is insignificantly below the general student average (80.177). The coefficient of variation (14.83) is also very slightly below the general student average (15.1). The coefficient of correlation between pulse rate and vital capacity in the present group (table 2), -0.161 ± 0.066, is insignificant, being less than three times the probable error.

Respiratory rate. The respiratory rate per minute averages 16.54 (range 8–25), with coefficient of variation 21.16 per cent (table 1). The coefficient of correlation between respiratory rate and vital capacity (0.173 ± 0.065) is insignificant, like that for pulse rate. The type of normal (quiet) respiration observed was purely costal (upper thoracic) in 1 case; purely abdominal in 43 cases; and mixed in 56 cases. Of the mixed cases, the abdominal respiration was predominant in 33 cases, the thoracic in 7, and neither in 16.

Breath-holding time. The average time the breath could be held voluntarily at the end of quiet respiration (expiration) is 33.7 seconds (table 1) with range of 14 to 96. After a maximum inspiration the average is increased to 72.37 seconds (range 34 to 176). After a maximum expiration, the average is reduced to 24.49 (range 11 to 75). The average difference between the breath-holding time at inspiration and at expiration is 47.92 seconds. The students showing the longest periods of breath-holding are those with previous experience in diving or playing wind instruments.

Theoretically, it might be expected that the time one could hold his breath would depend to a considerable extent upon the amount of air contained in his lungs, as indicated by the vital capacity. As shown in table 2, however, the correlations obtained in all cases are insignificant, even the highest coefficient (0.159 ± 0.066 for breath-holding at maximum expiration) being less than three times the probable error. The results are therefore definitely opposed to the conclusions of Wittig and Polczak (1926). It would appear that the time during which a person can voluntarily hold his breath must depend chiefly upon factors other than his vital capacity.

Chest girth. The average chest girth, measured at the nipple level at the

TABLE 1

Measurements on 100 male students

MEASUREMENT	MEAN AND PROBABLE ERROR	STANDARD DEVIATION AND PROBABLE ERROR	COEFFICIENT OF VARIATION AND PROBABLE ERROR
Vital capacity, cc	4406 ±39	575 ±27	13.05 ±0.63
Age, years	19.400 ±0.082	1.211 ±0.058	6.24 ±0.29
Stature, cm	173.08 ±0.41	6.02 ±0.29	3.48 ±0.14
Sitting height, cm	86.97 ±0.21	3.13 ±0.15	3.60 ±0.19
Sitting height^3, cm.^{3}	7543 ±37	547 ±26	7.22 ±0.34
Body weight, kgm	62.03 ±0.39	5.81 ±0.28	9.37 ±0.43
Pulse rate per minute	78.61 ±0.79	11.66 ±0.56	14.83 ±0.73
Respiratory rate per minute	16.54 ±0.24	3.50 ±0.17	21.16 ±1.04
Breath-holding, end of quiet resp., sec	33.70 ±0.89	13.14 ±0.63	39.0 ±2.1
Breath-holding, max. inspiration, sec	72.37 ±1.70	25.18 ±1.20	34.8 ±1.9
Breath-holding, max. expiration, sec	24.49 ±0.71	10.56 ±0.50	43.1 ±2.4
Breath-holding, inspir.—expir., sec	47.92 ±1.34	19.87 ±0.95	41.5 ±2.3
Chest girth (quiet), cm	84.53 ±0.28	4.19 ±0.20	4.96 ±0.24
Chest girth^3 (quiet), cm.^{3}	7163 ±51	749 ±36	10.45 ±0.48
Chest expansion, mm	72.47 ±1.13	16.82 ±0.80	23.21 ±1.15
Umbilical girth (quiet), cm	74.17 ±0.25	3.68 ±0.18	4.96 ±0.24
Umbilical girth (inspiration), cm	70.75 ±0.24	3.50 ±0.17	4.95 ±0.24
Umbilical girth (expiration), cm	72.50 ±0.24	3.54 ±0.17	4.88 ±0.24
Umbilical expansion, mm	−17.82 ±1.03	15.23 ±0.73	(36.7 ±2.1)
Epigastric girth (quiet), cm	74.72 ±0.26	3.91 ±0.19	5.23 ±0.24
Epigastric girth (inspiration), cm	80.31 ±0.29	4.24 ±0.20	5.28 ±0.24
Epigastric girth (expiration), cm	72.28 ±0.27	4.02 ±0.19	5.56 ±0.29
Epigastric expansion, cm	80.35 ±1.31	19.44 ±0.93	24.2 ±1.2
Trunk girth, average (quiet), cm	77.81 ±0.24	3.60 ±0.17	4.63 ±0.24
Trunk girth, average (inspiration), cm	80.16 ±0.24	3.59 ±0.17	4.47 ±0.19
Trunk girth, average (expiration), cm	75.65 ±0.24	3.57 ±0.17	4.71 ±0.24
Trunk girth, average, expansion, mm	45.07 ±0.77	11.39 ±0.54	25.3 ±1.3
Trunk girth, average, expansion^2, mm.^{2}	2164 ±70	1036 ±49	47.9 ±2.8
Thoracic index, percent	66.21 ±0.36	5.30 ±0.25	8.01 ±0.38
Weight : height^3 index	2.070 ±0.011	0.1623 ±0.0077	7.84 ±0.38
Weight : height^3 index	1.1983 ±0.0073	0.1088 ±0.0052	9.08 ±0.43
Weight : sitting height^3 index	9.421 ±0.055	0.818 ±0.039	8.69 ±0.43
Surface area (DuBois formula), m^2	1.7310 ±0.0066	0.0975 ±0.0047	5.64 ±0.29
Surface area (Dreyer formula), m.^{2}	2.820 ±0.013	0.1911 ±0.0091	6.78 ±0.34
Sitting height × chest girth, cm.^{2}	7356 ±36	529 ±25	7.20 ±0.34
"Trunk area" (quiet), cm.^{2}	6772 ±31	465 ±22	6.87 ±0.34
"Trunk area" (inspiration), cm.^{2}	6977 ±32	475 ±23	6.81 ±0.34
"Trunk area" (expiration), cm.^{2}	6584 ±30	446 ±21	6.77 ±0.34
"Trunk area" expansion, cm.^{2}	392.8 ±7.0	104.3 ±5.0	26.6 ±1.4
"Trunk volume" (quiet), cc	42,038 ±313	4636 ±221	11.03 ±0.53
"Trunk volume" (inspiration), cc	44,616 ±333	4942 ±236	11.08 ±0.53
"Trunk volume" (expiration), cc	39,741 ±300	4450 ±212	11.20 ±0.53
"Trunk volume" expansion, cc	4875 ±91	1346 ±64	27.60 ±1.44
Lundsgaard "chest volume" (quiet), cc	7171 ±65	968 ±46	13.50 ±0.63
Lundsgaard "chest volume" (inspiration),cc	8688 ±84	1251 ±60	14.40 ±0.68
Lundsgaard "chest volume" (expiration), cc	6678 ±59	882 ±42	13.21 ±0.63
Modified Lund. "chest volume" (inspiration), cc	10,338 ±102	1509 ±72	14.59 ±0.73
Lundsgaard "chest volume" expansion, cc	2011 ±36	540 ±26	26.9 ±1.4
Modified Lund. "chest volume", expansion, cc	3661 ±64	947 ±45	25.9 ±1.3
Ratio of vital capacity to:			
Lundsgaard "chest volume" (quiet), per cent	61.80 ±0.41	6.14 ±0.29	9.94 ±0.48
Lundsgaard "chest volume" (inspiration), per cent	51.05 ±0.35	5.12 ±0.24	10.02 ±0.48
Lundsgaard "chest volume" (expiration), per cent	66.40 ±0.48	7.07 ±0.34	10.64 ±0.53

TABLE 2

Correlations between various measurements on 100 male students

MEASUREMENTS CORRELATED	COEFFICIENT OF CORRELATION $r \pm$ P.E.	CORRELATION RATIO (η)
Age and stature	−0.058 ±0.067	0.183
Age and body weight	0.217 ±0.064	0.287
Stature and body weight	0.587 ±0.044	0.650
Vital capacity and:		
Age	0.041 ±0.067	0.319
Stature	0.666 ±0.038	0.711
Sitting height	0.574 ±0.045	0.631
Sitting height[2]	0.579 ±0.045	0.633
Body weight	0.743 ±0.030	0.773
Pulse rate	−0.161 ±0.066	0.285
Respiratory rate	0.173 ±0.065	0.298
Breath-holding time (quiet)	0.101 ±0.067	0.344
Breath-holding time (inspiration)	0.117 ±0.067	0.317
Breath-holding time (expiration)	0.159 ±0.066	0.316
Breath-holding time (inspir.—expir.)	0.099 ±0.067	0.301
Chest girth (quiet)	0.622 ±0.041	0.701
Chest girth[2] (quiet)	0.653 ±0.039	0.735
Chest expansion	0.328 ±0.060	0.481
Umbilical girth (quiet)	0.578 ±0.045	0.618
Umbilical expansion	0.053 ±0.067	0.316
Epigastric girth (quiet)	0.582 ±0.045	0.683
Epigastric expansion	0.365 ±0.058	0.580
Average trunk girth (quiet)	0.658 ±0.038	0.728
Average trunk girth (inspiration)	0.745 ±0.030	0.786
Average trunk girth (expiration)	0.618 ±0.042	0.677
Average trunk girth expansion	0.383 ±0.058	0.537
Average trunk girth expansion[2]	0.424 ±0.055	0.544
Thoracic index	0.005 ±0.067	0.354
Weight:height[2] index	0.328 ±0.060	0.453
Weight:height[3] index	0.025 ±0.067	0.034
Weight:sitting height[3] index	0.105 ±0.067	0.394
Surface area (DuBois formula)	0.791 ±0.025	0.812
Surface area (Dreyer formula)	0.760 ±0.029	0.789
Sitting height × chest girth	0.747 ±0.030	0.775
"Trunk area" (quiet)	0.757 ±0.029	0.778
"Trunk area" (inspiration)	0.797 ±0.025	0.812
"Trunk area" (expiration)	0.740 ±0.030	0.752
"Trunk area" expansion	0.458 ±0.053	0.590
"Trunk volume" (quiet)	0.739 ±0.031	0.776
"Trunk volume" (inspiration)	0.797 ±0.025	0.810
"Trunk volume" (expiration)	0.721 ±0.032	0.741
"Trunk volume" expansion	0.566 ±0.046	0.664
Lundsgaard "chest volume" (quiet)	0.701 ±0.034	0.759
Lundsgaard "chest volume" (inspiration)	0.718 ±0.033	0.754
Lundsgaard "chest volume" (expiration)	0.703 ±0.034	0.773
Modified Lund. "chest volume" (inspiration)	0.791 ±0.025	0.808
Lundsgaard "chest volume" expansion	0.544 ±0.047	0.594
Modified Lund. "chest volume" expansion	0.653 ±0.039	0.704

end of quiet respiration, is 84.53 cm.; coefficient of variation 4.96 per cent (table 1). These figures are slightly below the corresponding general student averages (88.54 and 5.99, respectively). The coefficient of correlation between chest girth (quiet) and vital capacity in the present group is 0.622 ±0.041 (table 2). This is considerably higher than the corresponding correlation (0.413 ±0.016) found by Cripps, Greenwood and Newbold (1923) in the larger group of Air Force candidates. The coefficient of correlation between the square of the chest girth (used by Dreyer) and the vital capacity in the present group is 0.653 ±0.039, which is insignificantly higher than the above-mentioned correlation for the chest girth.

Chest expansion. The chest expansion, or difference in chest girth between inspiration and expiration at the nipple level, ranges from 35 to 120 mm., with average of 72.47 mm. (table 1). The coefficient of variation is 23.21 per cent. These figures are slightly below the general student average (73.76 mm. and 28.75 per cent, respectively). The coefficient of correlation between the chest expansion and the vital capacity in the present group (0.328) is also slightly below that previously found for the larger student group (0.351). This emphasizes the conclusion that the chest expansion in general forms a poor index of the vital capacity (Jackson, 1927). Nevertheless, the chest expansion is still widely used by the medical profession as an indication of pulmonary capacity in examinations for life insurance, etc., although its unreliability for such purposes has been pointed out by various authors.

Umbilical girth. Chest expansion alone might be expected to give a poor indication of pulmonary (vital) capacity, since it measures only the costal respiratory movement and neglects the important factor of abdominal or diaphragmatic respiration. It would therefore seem probable that a better correlation with vital capacity might be obtained if the abdominal girth were also included. As shown by table 1, the means for the umbilical girth are 74.17 cm. at the end of quiet respiration, 70.75 cm. at maximum inspiration, and 72.50 cm. at maximum expiration. The corresponding coefficients of variation (4.96, 4.95 and 4.88) are nearly identical with that for (quiet) chest girth (4.96). The coefficient of correlation between the umbilical girth (quiet) and vital capacity (table 2) is 0.578 ±0.045, which is but insignificantly lower than the corresponding correlation between (quiet) chest girth and vital capacity (0.622 ±0.041).

The *umbilical expansion* is measured (like chest expansion) by subtracting the umbilical girth at maximum expiration from the umbilical girth at maximum inspiration. In most cases (90 out of the 100), this gives a *negative* value, because the abdominal wall in the umbilical region is usually contracted during forced inspiration. The individual values for the "expansion" vary from −68 mm. to +23 mm., with a mean of −17.82 mm. In calculating the coefficient of variation in a series like the umbilical

expansion, containing both negative and positive numbers, an erroneously exaggerated coefficient will be obtained by the usual method of computation. For the present series of umbilical expansions, this would give a mean of —17.82 mm., with standard deviation 15.40 mm., which would make the coefficient of variation about 86 per cent. In order to get a better measure of variability in the expansion series, the scale of measurements was arbitrarily decreased 24 millimeters, so that all the measurements in the series were negative in sign. By this procedure, the standard deviation remained practically the same, but the mean became —41.5 mm., which reduced the apparent coefficient of variation to about 36.7 per cent, as given in table 1. Even by this method, the umbilical expansion still appears distinctly more variable than the chest expansion, the coefficient for the latter being 23.21 per cent for the present group. The coefficient of correlation between the umbilical expansion and the vital capacity is insignificant (0.053 $\pm$0.067). This shows that the umbilical expansion *alone* is of no value as an index of vital capacity.

Since the abdominal (umbilical) girth is usually decreased at maximum inspiration (which is easily verified by inspection of the nude body, as was originally noted by John Hutchison), it is an interesting problem to account for the disposal of the abdominal viscera, which have been simultaneously displaced downward by the contraction of the diaphragm. The decrease in umbilical girth and the simultaneous contraction of the diaphragm during inspiration actually crowd the viscera into the epigastric zone, where a marked expansion is shown by inspection and measurements.

Epigastric girth. In addition to the chest girth and the umbilical girth, it is therefore necessary to measure also the epigastric girth (taken midway between the other two), since this region is affected by both the costal and the diaphragmatic or abdominal movements. The means for the epigastric girth (table 1) are 74.72 cm. at the end of quiet respiration, 80.31 cm. at maximum inspiration, and 72.28 cm. at maximum expiration. The corresponding coefficients of variation, 5.23 to 5.56, are slightly higher than those for the chest and umbilical girths. The coefficent of correlation between the (quiet) epigastric girth and the vital capacity is 0.582 $\pm$0.045, which does not differ significantly from that found for the chest and umbilical girths.

The *epigastric expansion* (girth at inspiration minus girth at expiration) is relatively large, for the reasons above stated. As shown in table 1, the range is 44 to 121 mm., with mean of 80.35 mm. It thus averages distinctly greater than the chest expansion (72.47 mm.), but its coefficient of variation (24.19) is not significantly different. The coefficient of correlation between the epigastric expansion and the vital capacity (table 2) is 0.365 $\pm$0.058, which is slightly (though insignificantly) higher than that for chest expansion and the vital capacity (0.328 $\pm$0.060).

Average trunk girth. Because all three trunk girths (chest, umbilical and epigastric) are simultaneously affected by respiration, the average of the three was also computed (table 1). At the end of quiet respiration, the mean for the average trunk girth (quiet) is 77.81 cm.; at inspiration, 80.16 cm.; and at expiration, 75.65 cm. The coefficients of correlation between these average trunk girths and vital capacity (table 2) are as follows: at end of quiet respiration, 0.658; at inspiration, 0.745; and at expiration, 0.618. These correlations (especially at inspiration) are somewhat higher than those obtained by using each of the three girths separately.

The average *trunk girth expansion*, however, might reasonably be expected to correspond to the vital capacity more closely than does the average trunk girth. The average trunk girth expansion ranges from 19 to 71 mm., with a mean of 45.07 mm. (table 1). Its coefficient of variation (25.28) is about the same as those for the chest and epigastric expansions, but less than that for the umbilical expansion. The coefficient of correlation between the average trunk girth expansion and the vital capacity (table 2), however, is only 0.383 $\pm$0.058, which is not significantly higher than that for the epigastric expansion alone (0.365 $\pm$0.058). The square of the average trunk girth expansion was also computed. Its coefficient of correlation with vital capacity (0.424) is but slightly higher.

Thoracic index. Several indices were also computed, in order to test the possible relationship between the vital capacity and the body build or form of the thorax. The thoracic index (ratio of sagittal to transverse diameter, expressed as percentage, at end of quiet respiration) ranges from 52 to 84, with mean of 66.21 (table 2). As shown in table 2, the coefficient of correlation (0.005 $\pm$0.067) indicates no correlation between the shape of the thorax and the vital capacity.

Body build. Three different indices of body build were computed. The *weight:height*^{2} *index* ranges from 1.76 to 2.46, with mean 2.07 (table 1). The coefficient of correlation between this index and vital capacity is 0.328 $\pm$0.060, which indicates a moderate degree of correlation. A somewhat lower degree of correlation (0.188 $\pm$0.016) was found by Jackson (1927) in the larger student group. The *weight:height*^{3} *index* in the present group ranges from 0.98 to 1.46, with mean 1.1983. The coefficient of correlation in this case (0.025 $\pm$0.067) is insignificant. The *weight:sitting height*^{3} *index* averaged 9.421. The correlation of this index with vital capacity (0.105 $\pm$0.067) likewise appears insignificant. In general, the body build is evidently not closely correlated with the size of the vital capacity.

Surface area. The surface area was estimated by two different methods. Using the Boothby-Sandiford nomogram, based on the formula of DuBois (1916) for height and weight, the surface area in the present group ranges from 1.535 to 2.015 square meters, with mean 1.731 (table 1). The coefficient of correlation between this surface area and vital capacity is 0.791

± 0.025. The surface area estimated according to Dreyer's (1919) formula (weight $^{0.72}$) appears greater, with a mean of 2.820 sq. m. The corresponding coefficient of correlation, 0.760 ± 0.029, is only slightly (insignificantly) below that for the surface area by the DuBois formula. It will be noted that the coefficient of correlation with vital capacity appears slightly higher for surface area than that obtained for any other character previously mentioned.

"Trunk area." Dreyer (1919) also used the product of the chest girth and sitting height, which might be considered as roughly proportional to the real trunk area. This product in the present series averages 7356 sq. cm. (table 1), and its coefficient of correlation with vital capacity is 0.747 (table 2). The "trunk area" in this series was also computed as the product of the sitting height and the average trunk girth. The means for this "trunk area" (table 1) are: at quiet respiration, 6772; at inspiration, 6977; and at expiration, 6584 sq. cm. The corresponding coefficients of correlation from these "trunk areas" with vital capacity (0.757, 0.797 and 0.740) are practically equal to those for surface area (0.791 and 0.760).

In this case likewise it might be supposed that the *expansion* of the "trunk area" (inspiration minus expiration) would correspond more closely to the vital capacity, so this was computed (table 1). Here again, however, as was found for the trunk girth expansions, the result is disappointing, since the correlation between "trunk area" expansion and vital capacity drops to 0.458.

"Trunk volume." A possible explanation of the failure to obtain better correlation between vital capacity and trunk girths or areas might be that incommensurable quantities are being compared. It would seem more rational to compare vital capacity with body volume, especially chest volume, as proposed by Lundsgaard. For this purpose, a series of arbitrary "trunk volumes" was obtained by computing in each case the volume of a cylinder with height equal to the sitting height and circumference equal to the average trunk girth. The corresponding "trunk volumes" (means) are: at quiet respiration, 42,038 cc.; at maximum inspiration, 44,616; and at maximum expiration, 39,741 (table 1). The corresponding coefficients of correlation between these "trunk volumes" and vital capacity (table 3) are 0.739, 0.797 and 0.721, which are nearly the same as for the corresponding "trunk areas."

The *"trunk volume" expansion* (table 1) is obtained by subtracting the volume at expiration from that for inspiration. The mean is 4875 cc., and the coefficient of correlation between the "trunk volume" expansion and the vital capacity (table 2) is 0.566 ± 0.046. This is somewhat higher than the correlation obtained for the "trunk area" expansion, but is still below the correlations for the corresponding "trunk volumes." It will be

noted that although the correlation is not very high, the mean "trunk volume" expansion is very close to the actual mean vital capacity (4406 cc.).

Lundsgaard's "chest volume." This arbitrary "chest volume" is obtained by multiplying together the sternal length and chest diameters (sagittal and transverse). The mean volumes (table 1) are: at end of quiet respiration, 7171 cc.; at maximum inspiration, 8689 cc.; at maximum expiration, 6678 cc. The corresponding coefficients of variation are 13.5, 14.4 and 13.2. The corresponding coefficients of correlation with vital capacity (table 2) are 0.701, 0.718 and 0.703, which are slightly (but insignificantly) below those for the "trunk areas" and "trunk volumes."

The average ratio of the vital capacity to the Lundsgaard "chest volume" in this series is: at quiet, 61.8 per cent; at inspiration, 51.05 per cent; at expiration, 66.4 per cent (table 1). The corresponding coefficients of variation are 9.94, 10.02 and 10.64. In their smaller series of 18 adults, Lundsgaard and Van Slyke found that the vital capacity averaged about 45 per cent of the "middle chest volume" (table 6). The variability of this ratio was 11.6 per cent in 27 cases observed by Lundsgaard and Schierbeck.

It will be noted that the Lundsgaard formula for "chest volume" at inspiration uses the same factor for the chest (sternal) length as at expiration. An attempt was made to correct this by adding to the sternal length at expiration a sum equal to the epigastric expansion plus the (usually negative) umbilical expansion. This sum should roughly measure the elongation of the thoracic cavity produced by the contraction of the diaphragm in inspiration. This "modified Lundsgaard chest volume" at inspiration is increased to an average of 10,338 cc. (table 1). The corresponding coefficient of correlation between this modified "chest volume" and vital capacity (table 2) is somewhat higher (0.791 ± 0.025), but still not significantly above the correlation obtained with the "trunk areas."

The *Lundsgaard "chest volume" expansion* (inspiration minus expiration) averages 2011 cc. (table 1) and its coefficient of correlation with vital capacity is 0.544 ± 0.047 (table 2). The *modified* Lundsgaard "chest volume" expansion ranges from 1900 to 5900 cc., with mean of 3661 cc. (table 1). Its coefficient of correlation with vital capacity (table 2) is 0.653 ± 0.039, thus failing to reach the degree of correlation shown by the "chest volumes" and "trunk areas." These like the preceding series of expansions therefore unexpectedly and unaccountably fail to give the higher degrees of correlation with vital capacity which theoretically ought to appear.

Comparisons. The chief results of the present study are apparent in the accompanying table, by comparing the degrees of correlation between vital capacity and various physical measurements, including those previously used for this purpose and a few typical new measurements among those which were tested. In making comparisons, however, it should be noted that the probable error of the difference between two statis-

tical measurements is equal to the square root of the sum of the squares of the probable errors of the two measurements. Furthermore, a difference is not considered as statistically significant if it is less than three times the probable error of the difference.

Correlation of vital capacity in 100 male university students with:

Measurements previously used:

	r ± P.E
Surface area (DuBois)	0.791 ±0.025
Surface area (Dreyer)	0.760 ±0.029
Chest girth × sitting height (Dreyer)	0.747 ±0.030
Body weight	0.743 ±0.030
"Chest volume" (Lundsgaard, expiration)	0.703 ±0 034
Stature	0.666 ±0.038
Chest girth (quiet)	0.622 ±0.041
Sitting height	0.574 ±0.045
Chest expansion	0.328 ±0.060
Breath-holding time (at expiration)	0.159 ±0.066

New measurements:

"Trunk volume" (inspiration)	0.797 ±0.025
"Trunk area" (inspiration)	0.797 ±0.025
"Chest volume" (mod. Lundsgaard insp.)	0.791 ±0.025
Average trunk girth (inspiration)	0.745 ±0.030
Expansion of "chest volume" (mod. Lund.)	0.653 ±0.039
Expansion of "trunk volume"	0.566 ±0.046
Expansion of "trunk area"	0.458 ±0.053
Expansion of average trunk girth	0.383 ±0.058
Weight:height^2 index	0.328 ±0.060
Respiratory rate	0.173 ±0.065
Thoracic index	0.005 ±0.067
Pulse rate	−0.161 ±0.066

SUMMARY

The results of the present study may be summarized briefly as follows:

1. In a selected group of 100 healthy students, numerous measurements were carefully taken (at inspiration, expiration and quiet respiration) in order to study the correlations between vital capacity and various physical characters. These characters included those hitherto in general use for this purpose, together with a large series of new measurements.

2. Of the older methods, the surface area apparently gives the best correlation with vital capacity in the present series. This correlation, however, is not significantly higher than those for body weight, stature, chest girth or Lundsgaard's "chest volume". The correlation for sitting height is somewhat lower. That for chest expansion is very much lower, although this measure is still widely used in physical examinations. The correlation between breath-holding time and vital capacity is insignificant.

3. The new measurements, whose correlation with vital capacity was tested in the present study, may be classified in three groups. The first group includes the "trunk areas," "trunk volumes" and trunk girths, which in general give correlations as good as (but not significantly better than) the best of the measurements hitherto used for this purpose. The second group includes the various expansions, which theoretically ought to give better correlations with vital capacity, but which actually yield unexpectedly and unaccountably lower coefficients. The third group includes the pulse and respiratory rates, and also the indices of body build, which (excepting the weight : height^2 index) give only insignificant correlations.

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PERSONAL CHARACTER OF THE PROLONGED STANDING CIRCULATORY REACTION AND FACTORS INFLUENCING IT

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Received for publication June 16, 1928

A paper published in THIS JOURNAL (Turner, 1927b) described a test used to judge the efficiency of the circulation. This test depends on the alteration of the circulation in quiet standing as compared with reclining, shown by the work of Field and Bock (1924), by Lawrence, Hurxthal and Bock (1927), and by the author (1927a). The findings, briefly, are 1, the lessening of the circulatory minute volume in quiet standing as compared with reclining in almost all the persons studied; 2, the frequent occurrence of progressive decrease in circulatory minute volume as standing is prolonged, and 3, the suggestion that circulatory minute volume is better maintained in those who show no history of faintness or dizziness when standing. A simple procedure for determinations of pulse rate and pressures in prolonged standing seemed (Turner, 1927b) to offer a way of differentiating those whose circulatory minute volumes fall off extensively from those who are able to maintain a circulatory adjustment adequate for the difficult task of standing still.

If such a test is of real significance and usefulness, it must be shown 1, that a given individual reacts under the same conditions in a relatively constant manner; 2, that different individuals show results which are sufficiently different from one another to make the test suggestive of relative success or failure in the circulation, and 3, that the variations which appear in a single individual's reactions under different circumstances are intelligible.

It is the purpose of this paper to state the experiments which were made to determine these points. The routine of the test was that formerly described, namely, a preliminary Schnedier test by the usual technic, and then the test particularly under study which consisted of frequent determinations of pulse-rate and arterial pressures during a period of reclining long enough to establish a steady state, followed by fifteen minutes of *quiet* standing and that in turn by about eight minutes of reclining as a final control.

The subjects were all young women between twenty and thirty years of age who were spending the summer at the Marine Biological Laboratory, Woods Hole, Mass., or students or laboratory instructors at Mount Holyoke College. They all considered themselves in good health. Some subjects were distinctly athletic, others were chosen because they had a history of occasional dizziness or faintness on standing, others were "ordinary" young women. No subjects were unusual in bodily proportions.

To establish the dependability of the circulatory adjustments in prolonged and quiet standing each subject was given the test six or more times

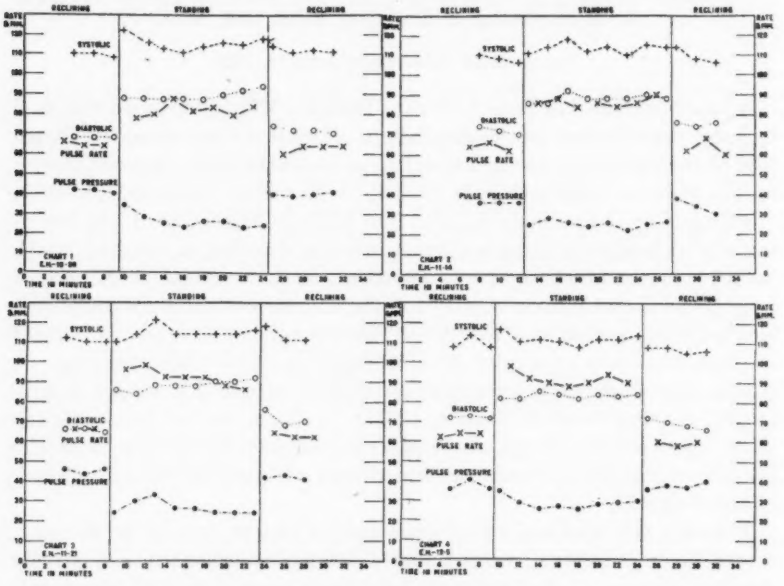

Fig. 1. Charts 1 to 4. Records of four prolonged standing tests on a single subject, E. H., to show degree of similarity in tests made on different days but under the same conditions.

under conditions as nearly the same as possible, at the same hour of the day, at a time well removed from the last meal, with no *vigorous* exercise during about two hours preceding the test. The subjects were undisturbed, the room was comfortable. In a reaction as flexible as circulatory adjustment the necessity of making the tests under conditions as rigidly controlled as possible cannot be emphasized too strongly. The first test was always discarded unless it was indistinguishable from later tests.

It was found that a given subject has a characteristic mode of reaction to quiet standing as compared with reclining. She may show a rise or a fall

or no change in systolic pressure, but she shows much the same change on successive days. She may show a slight or a pronounced increase in heart rate, and this again occurs day after day. Her pulse pressure may be characteristically high or low; she may maintain a steady state or show a progressive change as standing is prolonged; or a steady state may contrast with large random fluctuations, but whatever her reaction, it reappears day after day if conditions are the same.

The degree of likeness in successive tests is shown by figure 1, in which four tests are charted. It will be seen that subject E. H. (who is a college

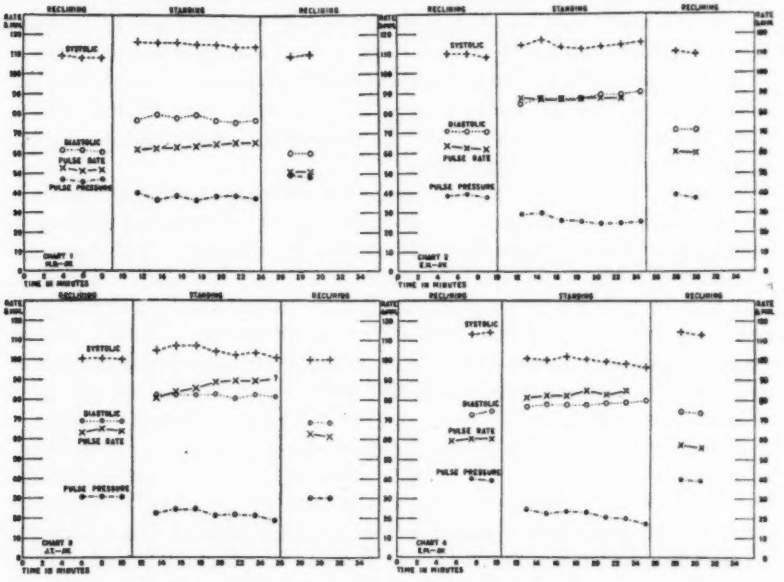

Fig. 2. Charts 1 to 4. Averages of six or more tests on each of four subjects. Tests of each series made under the same conditions. Note differences between charts, characteristic of individual subjects. Compare chart 2 with figure 1.

student of excellent carriage, successful in college athletic contests calling for both speed and endurance) has a circulatory reaction characterized by a reclining pulse of 60 to 65 which rises to nearly 90 on standing. The first two minutes after rising are in all tests of little importance since they reflect too readily the varying muscular work with which the subject happens to get up. The pulse rate of about 90 is well maintained to the end of the fifteen minutes of standing. Her systolic pressure rises about 5 mm. and does not fall as time goes on. The diastolic pressure rises 15 or 20 mm. and is maintained. The pulse pressure therefore falls, but is kept during

standing at about 25 mm., a perfectly safe level. These four tests with two others were averaged to give the second chart in figure 2. This chart, obtained by averaging comparable readings on the six separate charts, gives the characteristics of the individual records with minor fluctuations wiped out.

Three other average charts are shown in figure 2. It will be seen that each is individual. M. D. shows a slower pulse, with less rise than E. H. This, with her higher pulse pressure, is interpreted to indicate a more adequate heart muscle. J. T. has a history of dizziness on standing. Because of this she was frequently unable to stand for the usual 15 minutes, though this series at a favorable time shows only one such failure. Attention is called to her progressively rising pulse rate through the standing period, to the falling tendency in the systolic pressure and to the rather low pulse pressure. E. M. shows a large fall in systolic pressure, and also in pulse pressure, both of which tend to be progressive. She also has a history of habitual discomfort in standing.

These are representative of the ten young women studied in this way. Table 1 shows the findings for all of them, scored as described in the previous paper (Turner, 1927b) with notes as to their history. For comparative purposes, scores by Schneider's tests are also included. It will be seen that there is a general agreement though the scores of the Prolonged-Standing test run lower and there are a few exceptions. Though subject D. S. said she experienced some dizziness her circulatory findings fail to indicate it. She herself associated this with her eyes. She did not grow pale or have to lie down before the end of the fifteen minutes of standing.

It will be seen that the history of dizziness is confined to those whose scores are relatively low. By the Schneider test, however, two of the last five, D. S. and J. T., give scores as good as some of the subjects well above them in the prolonged standing reaction. But both of these subjects showed progressively unfavorable reactions as standing continued. J. T. always gave a good adjustment for a brief time and so could get a high Schneider score but could not maintain this state.

In addition to this group of ten subjects six others have been tested three or more times with a similar constancy in results. Their records also are included in table 1. Of these, E. P. is markedly individual because she shows a slight *fall* in pulse rate on first standing and later only a very slight rise, 2 to 4 beats per minute.

These patterns of circulatory adjustment have been evaluated according to the usually accepted criteria applied to rate and pressure findings. For the conditions of study already described, the best standing reaction for young women would seem to be as follows:

1. A pulse rate which rises from a reclining rate of from 50 to 60 by about 10 beats.

TABLE 1

Scores for Schneider and prolonged-standing tests for ten subjects, each tested six or more times under uniform conditions and for six other subjects tested three or more times

SUBJECT	NUMBER OF TESTS	SCHNEIDER SCORES	AVERAGE OF SCHNEIDER SCORES	PROLONGED-STANDING SCORES	AVERAGE OF PROLONGED-STANDING SCORES	REMARKS
M. D.	6	17,15,16,17,16,17	16.3	15,11,12,14,12,12	12.7	No dizziness
M. F.	8	18,18,18,18,18,17,18,18	17.9	10,11,14,11,12,12,13,14	12.1	No dizziness
E. K.	7	14,14,18,15,9,15,13	14.0	11,13,12,9,10,11,8	10.6	No dizziness
P. H.	8	12,9,9,8,12,11,8,14	10.4	15,10,8,8,11,12,8,10	10.25	No dizziness
E. H.	6	17,16,13,13,12,12	13.8	11,8,11,10,9,12	10.2	No dizziness
D. S.	6	10,15,14,13,12,18	13.7	5,7,9,9,12,11	9.2	Slight dizziness
R. H.	6	7,12,12,10,8,10	9.8	6,11,10,8,7,10	8.7	No dizziness except before breakfast
J. T.	8	13,13,13,14,12,10,10,12	12.1	9,4,8,9,−5,9,3,9	5.75	Often dizzy standing. One test incomplete because of this
A. D.	7	4,7,7,8,4,6,3	5.6	8,6,3,3,6,7,5	5.4	Score may be too high. Did not stand still, uncomfortable
E. M.	7	5,9,9,10,8,8,10	9.1	−1,−1,3,10,−2,3,9	3.0	Often dizzy standing. Uncomfortable
C. C.	3	17,18,15	16.7	12,14,12	12.7	Never dizzy
C. H.	4	13,12,7,12	11.0	10,10,9,9	9.5	A.M. tests. No dizziness
C. H.	3	13,14,15	14.3	9,12,12	11.0	P.M. tests. No dizziness
E. P.	3	16,14,16	15.3	9,11,10	10.0	No dizziness
H. C.	4	13,8,11,11	10.75	13,7,8,11	9.75	No dizziness
M. C.	4	13,11,15,16	13.75	7,7,8,11	8.5	No dizziness
E. D. M.	4	7,11,9,8	8.75	2,7,5,4	4.5	Wide fluctuations

2. A rise in systolic pressure.

3. A rise in diastolic pressure which however is not great enough to lower the pulse pressure unduly.

4. A pulse pressure not unduly low in standing, as much as 25 to 30 mm., certainly not under 20 mm.

5. The maintenance of all these conditions without large fluctuations or progressive change.

Unfavorable findings which seem to reflect a failure on the part of either the heart or the vasomotor adjustment are as follows:

1. A high reclining rate. A large increase on standing. Further increase or abrupt fall as standing is continued. The last is usually found to precede or to accompany acute dizziness or fainting.

2. A fall in systolic pressure, either throughout standing or after an initial rise. A marked fall in systolic pressure is frequently found by the observer before dizziness or fainting is felt subjectively.

3. A marked and progressive rise in diastolic pressure. This may make the pulse pressure very low and indicate insufficient heart output. The diastolic pressure may fall abruptly before acute dizziness or fainting.

4. A low pulse pressure. A pulse pressure of above 20 mm. is seldom accompanied by dizziness. Some young women stand successfully for 15 minutes with pressures lower than 20 mm., but nearly all such have histories of poor circulatory adjustments of some sort.

5. Large fluctuations in either rate or pressures which seem to reflect circulatory instability.

Some subjects have been under observation for a long time. M. F.'s results extend over fifteen months, those of two others have been taken after two years, with little variation.

It is thus clear that circulatory adjustment to the demands of prolonged and quiet standing is effected by each person according to her own pattern, and that some patterns are far superior to others.

In attempting to judge the effect of variation in conditions upon a single individual, two types of variations are to be thought of: one, the variations in inner conditions occurring over a considerable period of time and together making up what we call the individual's "physical fitness"; and the other, such variations as occur in the ordinary day's program.

Of the former, no detailed study has been made since obviously the observer must await his opportunity. However, five interesting instances have appeared, all of which are suggestive. They are summarized in table 2.

M. C. was tested three times one year and four the following year. Before she knew her scores she was asked to compare her "general condition" for the two years. In 1926 she was working hard near the end of the college year, but hours of sleep and living conditions were regular and favor-

able. In 1927 there had been in the weeks immediately preceding the tests considerable travel with consequent irregular living conditions and lack of sleep. She thought herself better in 1926 in spite of hard work. Note the scores of both Schneider and Prolonged Standing tests. In 1927 the record is distinctly less good.

E. D. M. shows the opposite change, from a generally fatigued condition which had interfered with her work to a condition of fairly good health which permitted a full program. The better circulatory reaction is noteworthy.

TABLE 2

Scores for Schneider and prolonged-standing tests on five young women under circumstances affording contrasts in general physical fitness

SUBJECT	SCHNEIDER SCORES	AVERAGE OF SCHNEIDER SCORES	PROLONGED-STANDING SCORES	AVERAGE OF PROLONGED-STANDING SCORES	REMARKS
M. C.	13,11,15,16	13.75	7,7,8,11	8.5	March and June, 1926
	9,10,9	9.3	4,2,4	3.3	July and August 1927 Better condition 1926
E. D. M.	7,11,9,8	8.75	2,7,5,4	4.5	July and August 1927
	13,13,15	13.7	11,10,9	10.0	March 1928. Better condition 1928
H. C.	13,8,11,11	10.75	13,7,8,11	9.75	Intermenstrual
	11,8	9.5	−8,+6	−1.0	Menstrual
C. C.	17,18,15	16.7	12,14,12	12.7	Ordinary diet
	17,16,16,15	16.0	12,11,11,9	10.75	Reducing diet
C. H.	13,12,7,12	11.0	10,10,9,9	9.5	July, 1927. Woods Hole
	9,8	8.5	4,6	5.0	July 1925. Boston after prolonged hot weather

H. C. is a young woman who has marked dysmenorrhea. Throughout these tests the first two days of the menstrual period were systematically avoided for all subjects. This subject came in on the fourth day of the period and became too faint to continue standing. Pain was still being experienced. It was therefore decided to compare her intermenstrual and menstrual reactions, a matter of particular interest since so many women suffer from circulatory inefficiency as shown by fainting in connection with menstruation. Table 2 shows the average of four intermenstrual tests on H. C., two in each of two months and the results of two menstrual tests taken on the fourth day of two different periods. In the first of the men-

strual tests the subject became faint and unable to stand after the sixth minute, in the second test marked dizziness occurred though she stood for the whole fifteen minutes. At one point she would have fallen had she not been caught by the observer. Throughout the standing period of this test there was a very close relation between the subjective sensations of the subject and the changes observed from minute to minute in the pulse and pressures. The contrast between intermenstrual and menstrual reactions is marked.

C. C. presents a picture of nutritional contrasts. Three tests typical of a good circulatory reaction were followed by four successive tests much less favorable in their results. The observer found that the subject was "reducing" and had lowered her food intake from the customary 1800 to 2000 calories to 1000 to 1200. The findings in the circulation were exactly those of Benedict's subjects in prolonged fasting and in his group of men on a decreased ration (Benedict, 1907, 1915; Benedict, Miles, Roth and Smith, 1919): a slower reclining pulse, with a decreased pulse pressure. When the subject stood her pulse increase was greater than before, her systolic pressure rose less, her diastolic more, with a very low pulse pressure as a result. Her hands showed marked cyanosis, not noted in earlier experiments, but she did not get dizzy. However during this period of decreased food intake she failed on one occasion to be able to complete a basketball practise period and showed other indications of being less fit. Note her findings as shown in table 2.

C. H. shows a marked difference between two tests in 1925 and seven in 1927, the former taken after a long season of very hot weather when the subject had been in the city and had felt "depressed by the heat," the latter when she was at Woods Hole in moderate weather and apparently in excellent health. The hot weather records are inferior in that they show a reclining pulse of 75 instead of 60, a rise in standing of about 20 beats instead of 12 to 14, a slightly lower systolic level, with a tendency to fall on standing rather than to rise, a much greater diastolic rise on standing making the pulse pressure only 15 to 20 mm. instead of 25 mm. or more. The picture in hot weather may be due to the dilatation of skin vessels and a consequent failure to manage the standing adjustment successfully because of this large amount of blood to which no help is given in its return to the heart.

Thus, as far as these findings go, they indicate a parallelism between the results of this test and that elusive thing, fitness.

For the study of variations during the ordinary daily program, there are several interesting possibilities, the effect of food, exercise, fatigue, temperature and so on. Only the effect of food has been studied thus far and that with but two series of tests. Schneider and Truesdell (1923) have stated that the Schneider test gives lower ratings after meals largely because of the higher pulse rate at these times. The implication is that the circulation is

less adequate. However, ordinary experience goes to show that persons feel better after eating,—"Not able to work on an empty stomach," "All gone before dinner," "Can't start without breakfast,"—these are phrases frequently heard.

Thirteen pairs of prolonged standing tests were made at Woods Hole before and after the midday meal (dinner) under conditions otherwise similar. The effect of the meal on the *reclining* circulatory conditions was as follows. In each finding the condition after the meal is referred to that before the meal.

Increase in pulse rate, average 13.0 beats, ranging from +6 to +18 beats.
Increase in systolic pressure, average 3.7 mm., ranging from −2 to +11 mm.
Decrease in diastolic pressure, average 7.4 mm., ranging from −2 to − 19 mm.
Increase in pulse pressure, average 11.1 mm., ranging from +5 to +21 mm.

The picture is thus consistently one of greater circulatory minute volume, a more rapid heart with a wider pulse pressure. This greater circulatory minute volume appears to favor success in prolonged standing for while the scores of the tests after the meal showed no appreciable change, average 7.2 before and 7.1 after, a careful study of the results showed in all cases except one that the systolic change after the meal was as good or better than before, and that in all cases that important factor, pulse pressure, was better maintained. The change in pulse rate varied in individuals but in only four cases by more than six beats per minute. Three of these cases showed less rise in rate after the meal and one a greater rise. These results are all for the significant period, minutes 5 to 15 after rising.

Thus in general it is shown that the greater heart outflow due to the meal was maintained during standing and the reaction of the subject thereby favored. The subjects all agreed that it was "easier" to stand after than before the meal. No one of them had the slightest discomfort after the meal whereas before it one subject had to lie down from dizziness after seven minutes of standing, another complained of some dizziness, and three others showed a fall in systolic pressure. All these conditions disappeared after the meal. The average Schneider scores were 11.5 before and 8.0 after, a marked contrast to the maintenance of the scores in the Prolonged Standing test as given above, and to the improvement felt subjectively and noted above by the careful study of the records.

This improvement in circulatory adjustment in prolonged quiet standing due to the meal is related presumably to a better return of blood to the heart. The picture may be one of persistent massage by the movements of the alimentary tract, thus pushing the blood, now present in enlarged amounts in the digestive system, back toward the heart so abundantly that the supply is entirely adequate to overcome the unfortunate effects of gravity when the subject rises and stands still.

Another method of comparing the tests before and after a meal is given in figure 3, charts 1 and 2. In these charts rectangles are constructed with pulse pressures as their vertical diameters placed against a blood pressure scale to show systolic and diastolic levels. The horizontal diameters give the heart rate, 20 beats to a unit along the base line. The first rectangle shows the condition just before rising, after about 10 minutes in the reclining position; the second rectangle, the first reaction to standing, 2.5 minutes after rising; the third, the condition at the last standing determination, 13.5 minutes after rising; and the fourth, the condition 3 minutes after

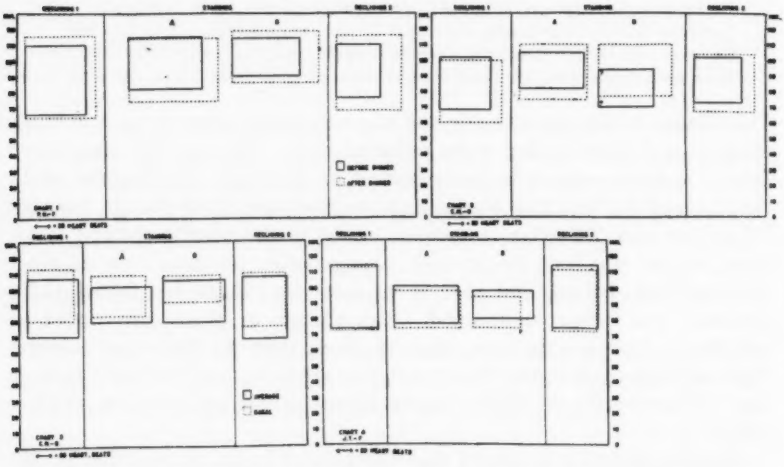

Fig. 3. Charts 1 and 2. Comparison of tests before and after dinner, other conditions the same. Vertical diameter of rectangles, pulse pressure extending between systolic and diastolic levels as shown by scale. Horizontal diameter, pulse rate, with one unit equal to twenty heart beats: Reclining 1, last determinations before rising. Standing A, 2.5 minutes after rising. Standing B, 13.5 minutes after rising. Reclining 2, 3 minutes after reclining. Charts 3 and 4. Comparison of test in basal condition with daytime average. Times as in charts 1 and 2.

reclining for the second time. Obviously these are based on the general idea that both heart rate and pulse pressure give testimony regarding the heart's output, though probably it is unwise to push too far the suggestiveness of Erlanger and Hooker's much discussed formula, Pulse Rate $\times$ Pulse Pressure, as indicating the relative volume of the output (Erlanger and Hooker, 1904).

As these figures are compared it will be noted that they show graphically, not only that the flow in standing is much greater after a meal than before but that conditions for prolonged standing are better maintained. Note

condition at minute 13.5 after rising. The greater volume of blood brought into circulation by the meal thus appears to favor the efficiency of the medullary centres even though we picture much of this extra supply as directed differentially to the digestive tract.

That this may be the true explanation of the state after meals is indicated further by another series of ten experiments performed on eight subjects

TABLE 3

Comparison of Schneider and prolonged-standing tests given under basal metabolic conditions with the day-time averages

SUBJECT	DAYTIME AVERAGE SCORE		BASAL SCORE, SINGLE TESTS		REMARKS ON CHANGE IN STANDING IN BASAL TESTS
	Schneider	Prolonged-standing	Schneider	Prolonged-standing	
M. F.	17.9	12.1	15	13	Subject eats little breakfast normally. Scores as good as for daytime but less sys. rise than usual, slightly lower pulse pressure, pulse rate rose progressively
			16	14	
R. H.	9.8	8.7	13	−6	Had to lie down at 9th minute of standing. Never dizzy at any other time of day. Sudden fall of pulse from very rapid rate
C. H.	11.0	9.5	14	7	Progressive diastolic rise, pulse pressure down to 16 mm. usually 24 mm. See figure 3
P. H.	10.4	10.25	10	10	Never eats breakfast. Test indistinguishable from late forenoon.
E. K.	14.0	10.6	12	7	Usual systolic rise missing, progressive diastolic rise; progressive rise in pulse rate; irregular pulse rate
E. M.	9.1	3.0	12	−9	Both basals terminated by dizziness at 10.5 minutes and 9 minutes with usual findings
			10	−8	
D. S.	13.7	9.2	14	10	Reaction much as usual; slight dizziness
J. T.	12.1	5.75	10	−8	Test terminated by dizziness after 10.5 minutes standing. See figure 3
Average...	12.25	8.6	15.75	3.75	

before breakfast when they were in basal metabolic condition. The findings are tabulated in table 3 and shown graphically in figure 3, charts 3 and 4, for one experiment. These basal findings confirm the before-and-after-dinner series in that they show circulatory reactions less good than when food has been taken, basal average 3.75, daytime average 8.6. It will be noted, however, that the average Schneider score is *better* before breakfast than later in the day, average 15.75 as compared with 12.25. Again this depends

mainly on the heart rate which is low in basal metabolic condition. Here also the prolonged standing test gives evidence more in harmony with daily experience in that breakfast tends to make most persons feel more fit.

The evidence derived from this test is thus at variance with Schneider's results on two points, relative failure in the basal condition and relative success after meals.

Further studies are in progress with respect to the changes in this reaction after exercise and when the body temperature has been altered. Thus far all of the work seems to reflect the general success of the individual in the adjustments of daily living and in a way explicable on the theory that the essential factor to be considered is an adequate return of blood to the heart. The relation of this to the nutritional program, both in daily meals and in general nutritional level, appears worthy of emphasis.

SUMMARY

1. The Prolonged Standing test devised to test circulatory efficiency was made on each of ten healthy young women six or more times under conditions as nearly the same as possible in respect to meals, time of day, and general activity preceding the test. On six others, shorter series of tests were made.

2. The results indicate that an individual under constant conditions shows a definite and dependable pattern of circulatory adjustment to the change from reclining to standing and also a constant degree of endurance in prolonged standing. These patterns differ markedly from one individual to another.

3. By using well-accepted criteria of circulatory success as shown by changes of pulse rate and arterial pressures these sixteen healthy persons have been graded to bring out their relative circulatory efficiency in prolonged standing.

4. It is essential for comparative results that the conditions of observation be carefully controlled, especially in regard to eating and general activity preceding the tests. Relatively slight changes have detectable effects.

5. Supplementary data from five other individuals indicate that when these tests are made at times when the general condition of the subject is known to vary, the results reflect the subject's physical success. Examples are given from two subjects who were in better health at one time than another, from one subject who showed marked dysmenorrhea, from one subject who was on a reducing diet, and from one subject whose condition had been physically lowered by prolonged hot weather.

6. A study of the effect of eating on the prolonged standing reaction was made by thirteen pairs of tests on subjects before and after the mid-day meal (dinner) under conditions otherwise the same. These showed without exception that the individuals were better able to maintain the circulation during prolonged standing after than before the meal.

7. Ten tests on eight subjects early in the morning under basal conditions showed them less able to maintain standing than under ordinary daytime conditions.

8. It is noted that the findings in 5, 6 and 7 are in harmony with the ordinary events of life, though in 6 and 7 not parallelled by the ratings given by Schneider's circulatory test. Emphasis is placed by these results on the maintenance of efficient circulatory responses by a suitable nutritional program.

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SOME CONDITIONS AFFECTING THE CAPACITY FOR PROLONGED MUSCULAR WORK

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Received for publication October 10, 1928

The remarkable growth of our knowledge of the functioning of muscle, that has been an outstanding feature of recent advances in physiology, has been based largely on studies of isolated muscles stimulated to exhibit single twitches or brief tetanic contractions. These new developments in muscle physiology, arising in the main from researches on isolated muscles, raise new questions as to the support and collaboration of other organs in the body. In physical exercise the heart beats more rapidly, the capillaries of the active muscles dilate, sugar is freed from the liver, adrenin is discharged into the blood stream. What is the effect on the fundamental operations in active muscles if these attendant conditions are disturbed? What happens if they are favored? These questions were the occasion for the research which is here reported.

METHODS. In order to test the influence of accessory organs on the capacity for muscular work, maximal endurance was used as a measure. Healthy dogs of medium size were trained to run in a treadmill.[2] The endless belt, inclined at an angle of ten degrees, was moved by an electric motor at a known speed which varied in different experiments from 110 to 206 meters per minute. The animals, which had not eaten for 18 to 20 hours, were exercised in this mill until in most cases they were thoroughly exhausted. This state was indicated by their lying down and refusing to run farther, by refusing to rise after the mill had been stopped even though the collar was pulled and encouraging sounds, such as whistling and calling, were made, and by promptly lying down, if they had been coaxed to their feet, and refusing to run when the mill was started again. Of course, the dog might soon learn that this was a way out of doing work and might resort to it in order to avoid disagreeable labor. In some

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[2] We take pleasure in thanking Dr. F. G. Benedict, of the Nutrition Laboratory of the Carnegie Institution of Washington, for generously lending us the treadmill which we used.

instances, indeed, the animal, when apparently excited, did refuse to run without being tired. As a rule, however, the trained dogs ran eagerly and only stopped when signs of fatigue (to be described later) became evident. After the animal had refused to run, he was permitted to rest a definite length of time, usually five minutes, whereupon the mill was started again. Thus the test was continued until the dog refused to run at all, and in some instances was so completely exhausted as to be unable to stand. At no time was there evidence of pain or sharp discomfort—any more than there is in runners or in members of a boat crew who strive to the limit of their endurance. When blood samples were taken at fairly regular intervals for estimating blood sugar and lactic acid in the course of a test, the treadmill was stopped only long enough to obtain the sample and to count the pulse and respiration—usually not longer than from three to six minutes.

In each experiment the time of each period of exercise, the speed of the moving belt and the pulse and the respiration at the end of the period were recorded. For training and also for determining standard maximum working capacity experiments were repeated on each animal until in successive trials the expenditure of energy was approximately the same.

The total expenditure of energy in excess of the basal metabolism was calculated for each experiment. In doing this we used factors representing the average of several determinations on dogs of different weights by Zuntz (1903) and by Slowtzoff (1903). By measuring metabolism they determined the energy expenditure under conditions similar to those in our experiments, except that the speed was somewhat less. Zuntz found the excess metabolism (for moving horizontally 1 kg. of the body 1 m.) was not increased in a dog weighing 29 kg. as its running rate rose from 57 to 101 m. per minute. It would appear that variations in effects of muscle viscosity (Hill, 1926) did not play an important rôle in these experiments. Most of our animals ran at the rate of about 124 m. per minute and maintained that rate for hours. It seems probable that within the range of our usual tests the energy output for a given distance was not much influenced by variations of speed. With variations of weight, however, conditions change. In dogs of different weight the energy expenditure per kilogram transported horizontally 1 m. varies considerably. In the experiments of Zuntz and Slowtzoff it averaged 0.67 kgm. The observations by Anderson and Lusk (1917) on a dog weighing 9 kg. yielded the figure 0.58 kgm. We have taken 0.6 kgm. as the energy expenditure for transportation of 1 kilogram of the dog through 1 meter of horizontal distance. The figures for vertical work are much more constant for animals of different weights than are the figures for horizontal progression. With elevations varying between 14 and 24 per cent and a horizontal speed from 24 to 27 m. per minute, Zuntz and Slowtzoff found that the vertical work was almost constant—

about 3 kgm. for each kilogram of body weight lifted 1 m. We have taken, therefore, 3 kgm. as the energy expenditure for lifting 1 kg. 1 m. The elevation used by us was 10 degrees, or nearly 18 per cent. The following formula has been used to calculate the energy expenditure per kilogram body weight in excess of the basal metabolism:

$$E = (0.6 \times a \times \cos. 10°) + (3 \times a \times \sin 10°) = a \times 1.11$$

in which a represents the running distance at 10° slope. The figure for the energy expenditure thus calculated is probably minimal. It approximately represents the work of transporting the body, but does not include that involved in the functioning of the respiratory muscles. Moreover the animals were exercised to fatigue or exhaustion; in such conditions the doing of muscular work is probably uneconomical and the energy expenditure for the transportation of the body may consequently be greater than is assumed above. And further, during the exercise the body temperature rose 1 to 2°C., and that might increase the metabolic rate by at least 10 per cent (Du Bois, 1924). All the dogs weighed about 10 to 12 kg.

The character of the performance of the animal during the experiment was judged and recorded. The following letters were used to designate his behavior:

V = vigorous running with rare stops.
S = steady running in the main with occasional lapses and stops.
I = irregular running with frequent stops, or stumbling and occasional refusal to run.
R = refusal to run for more than brief periods; incoördination; lying down and refusal to rise.

The following letters were used to indicate the condition of the animal at the end of the experiment:

A = active; ready to continue running.
W = weary; panting, but easily able to continue running.
T = tired; panting heavily, disinclined to run.
F = fatigued; panting very heavily, continues to run if urged.
E = exhausted; panting most heavily with head down, drops to platform and refuses to rise.

In a series of twelve experiments in which the maximal running capacity was established, eight were employed to test the effect of adrenalin. The drug was administered subcutaneously in doses varying from 0.02–0.17 mgm. per kg. of body weight. In a few experiments it was administered at the start.

In many instances before the start and at intervals during the experiment samples of blood (5 cc.) were taken from the jugular vein for sugar and for lactic acid determinations. The blood was deproteinized and the sugar was

estimated by the method of Folin and Wu (1919). In order to determine the lactic acid the sugar was removed from the filtrate with copper sulphate and calcium hydroxide according to Clausen (1922). The lactic acid was oxidized to acetaldehyde by the procedure of von Fürth and Charnass (1910) with the latest modifications by Embden (1925). The titration of the acetaldehyde in the distillate was made according to Clausen (1922) in bicarbonate solution with 0.005N iodine. In connection with this work about 250 determinations of blood lactic acid and about 500 of blood sugar were made, but only the characteristic findings will be reported in this paper.

TABLE 1

Details of four experiments on dog K, illustrating the method of testing the working capacity and showing the increase of capacity with training

The rate of running in each experiment was 124 meters per minute.

	EXPERIMENT I	EXPERIMENT II
Date	11-1-27	11-3-27
Work (min.)	22 9 7 2 0 = 40	36 7 4 0 = 47
Rest (min.)	5 5 5 5	5 5 5
Performance	V S S R R	V V I R
Condition at end	E	E
Energy (1000 kgm.)	6	7
	EXPERIMENT III	**EXPERIMENT IV**
Date	11-5-27	11-6-27
Work (min.)	83 5 12 10 4 0 = 114	78 21 8 3 0 = 110
Rest (min.)	5 5 5 5 5	6 5 5 5
Performance	V I V S I R	V V S S R
Condition at end	E	E
Energy (1000 kgm.)	16	15

RESULTS. *Increase of working capacity with training.* In table 1 a series of four experiments on dog K are recorded in detail, which illustrate the method employed in this research. It will be noticed in each instance that as the experiment progresses there is a marked decrease in the working time of the animal between rest periods. Finally he refuses to continue running. This marks the end of the experiment and is taken as a limit of the animal's capacity to work. Some animals appeared to be more completely exhausted at the end of test than did others and to that degree there may be error in judging the working capacity. The consistency of the performances, however, has convinced us that this is a minor error and that as a rule the animals work to the limit of their power. The character of the running during the course of the experiment and the condition of the animal

at the end are given in the table, along with the energy production in excess of the basal metabolism expressed in 1000 kilogrammeters.

TABLE 2

Records of two tests of each of twelve animals

The first in each instance represents the initial performance after preliminary training; the second represents the maximal performance after further training.

ANIMAL	EXPERIMENT NUMBER	WORK	RATE	PERFORMANCE	CONDITION AT END	ENERGY (1000 KGM.)
		minutes	*m./minute*			
A	I	113	110	I-S-I	T	14
	VII	275	121	S-I-S	W	37
B	I	78	129	I-I-I	E	11
	V	251	121	S-I-R	E	34
C	I	73	121	I-I-R	E	10
	IV	140	114	S-I-I	F	18
D	I	75	122	I-I-R	F	10
	IV	104	112	I-I-R	F	13
E	I	117	127	I-I-R	E	17
	III	145	122	I-S-I	F	20
F	I	83	123	S-I-R	E	11
	V	96	156	S-I-R	E	17
G	I	94	121	S-I-I	E	13
	IV	188	125	S-S-R	E	26
H	I	66	172	S-S-R	E	13
	II	75	206	S-S-I	E	17
J	I	46	110	V-V-I	E	6
	IV	55	112	V-V-I	E	7
K	I	40	124	V-S-I	E	6
	III	114	124	V-S-R	E	15
L	I	62	124	V-I-R	F	9
	III	167	124	V-V-R	E	23
M	I	35	124	V-V-I	F	5
	III	100	124	V-V-V	E	14

All the animals were given preliminary training to accustom them to the apparatus before the first recorded experiment was performed. As shown in table 1, the ability to work continues to improve thereafter. This phe-

nomenon was typical. The performance after a series of tests which revealed no further increase of work was taken to represent the maximal capacity of the trained animal. It was used as a standard with which to compare the effect of various experimental conditions on the capacity.

Table 2 presents summaries of tests on each of the twelve animals used in the course of this research. With each animal are recorded two tests; the first represents the initial record performance, the second represents the maximal performance of the same animal. In every instance there is a

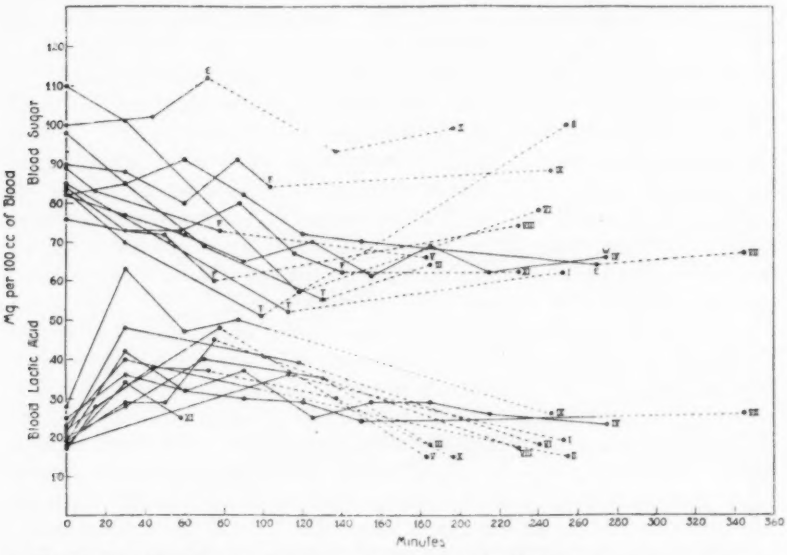

Fig. 1. Graphs showing the changes in the levels of blood sugar (upper group) and lactic acid (lower group) during work to exhaustion (solid lines) and after rest (dotted lines). For further description see text.

marked increased in the maximal over the initial performance, a difference which we attribute to training.

The pulse rose from a usual range between 75 and 100 beats per minute while the animals were at rest to a usual range between 155 and 190 during exercise. In any single experiment the rate remained at a fairly uniform high level during the period of exercise and returned to the basal within 15 to 30 minutes after the running ceased. There was some indication of a higher rate in the early stages of training than later. In dog A, for example, the pulse when the animal was resting quietly varied between 90 and 96 beats per minute, with 102 on one occasion. In the early tests the rate rose during work to 180 and 192, but in the later tests the maximum was between 156 and 168.

The relation of blood sugar and lactic acid to the work capacity. The results of the sugar and lactic acid determinations are reported as graphs (fig. 1) showing the levels at various times during the course of exercise and also after a period of recovery had elapsed after the exercise ended.

Figure 1 shows the results obtained in eleven experiments on four animals. The upper graphs represent the blood sugar, and the lower, the blood lactic acid values. The first portion of the graphs (in solid line) indicates the period of exercise and the latter portion (in dotted line) indi-

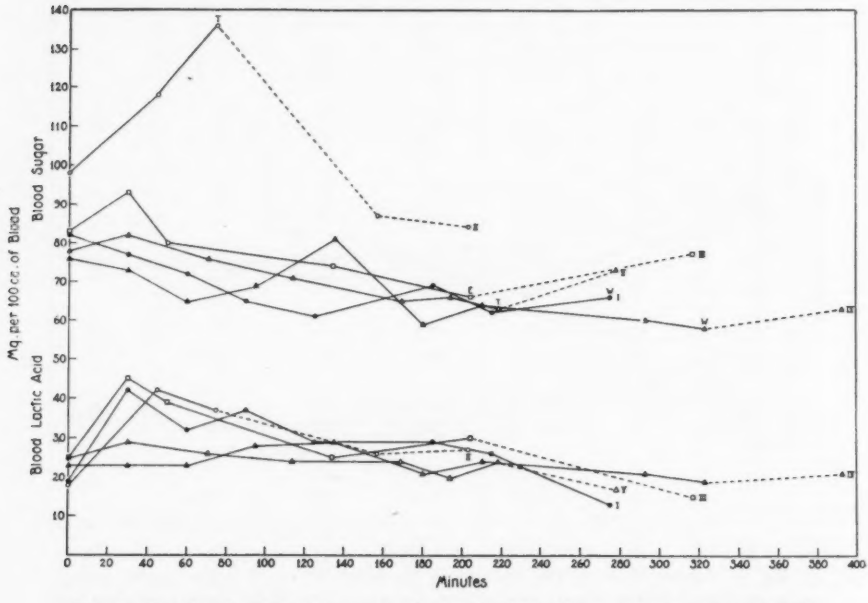

Fig. 2. Graphs showing changes in the level of blood sugar (upper group) and lactic acid (lower group) during work to exhaustion (solid lines) and after rest (dotted lines) in the same animal (dog A) under different conditions. Each mark in a line indicates an analysis. For further description see text.

cates the period of rest which followed. The letter at the end of the work period in each instance (see blood-sugar graphs) tells the condition of the animal at that time. The same Roman number at the ends of lines in the two groups of graphs signifies samples of the same blood for the two determinations.

The blood sugar in all except two experiments (VII and X) showed a decrease during exercise and an increase during the recovery. In one of the exceptional cases (VII) there was an initial rise followed by the usual decline during exercise and a rise during recovery; in the other (X) exhaustion

occurred when the blood sugar was at its highest level, and thereafter the level fell to below the initial value. As indicated by the letters over the curves there is no close correlation between the blood-sugar level and the condition of the animal; some were exhausted (E) when the level was relatively high (e. g., X), some were only tired (T) when it was low (e.g., II). This evidence is supported by tests on the effects of preliminary administration of glucose by stomach tube (see fig. 2). The blood sugar instead of falling rose during the exercise from 98 to 136 mgm. per cent. This abundance of available sugar, however, did not increase the amount of work done before the animal was exhausted. From all these observations we conclude that fatigue is not directly related to the level of blood sugar.

The lactic acid in all experiments rose during the first part of the exercise. This rise was followed by a decline which continued during the recovery period. The lactic acid level was higher at the end of exercise than at the beginning but in only two experiments (V and VIII) was it maximal when the animal was fatigued. As the figure shows, there was no close relation between the percentage of lactic acid and the condition of the animal. Furthermore, the improvement in the working capacity with training was not associated with any corresponding change in the lactic acid content of the blood. We conclude, therefore, that fatigue or exhaustion is not determined solely by the relative amount of lactic acid in the blood.

Some effects of giving glucose and adrenalin. Figure 2 presents the results of five experiments on dog A. Each mark in a line indicates an analysis. Line I with dots represents the normal; line II (circles) records an experiment in which 300 cc. of 10 per cent glucose was given by stomach tube ten minutes before start; line III (squares), an experiment in which 0.091 mgm. of adrenalin per kilogram of body weight was given subcutaneously three minutes before the start; line IV (solid triangles), an experiment seventeen days after the right adrenal was removed and the left denervated; and line V (hollow triangles), nine days after the left adrenal was demedullated. The average running rate lay between 117 and 121 m. per minute, except in the test represented by line V when it was 130.

Injection of adrenalin caused the blood sugar to rise from 83 to 93 mgm. per cent during the first 30 minutes of exercise, whereas without it there was a decrease from 82 to 77. The lactic acid also rose to a slightly higher level with adrenalin than without. Preliminary injection of glucose or adrenalin had no favorable effect on the amount of work done. Removal of the right adrenal and denervation and later demedullation of the left likewise did not produce any marked change in performance. There was, however, a noteworthy decrease in the accumulation of lactic acid in the blood during the exercise—the maximal figure being 29 mgm. per cent after 135 minutes of exercise instead of 42 mgm. after 30 minutes.

Figure 3 represents three experiments on dog B. Each mark in a line

indicates an analysis. Line 1 (dots) represents the normal performance at 121 m. per minute; line II (circles) records an experiment in which 0.174 mgm. of adrenalin per kg. was given 3 minutes before the start (average rate 115 m. per minute); line III (squares), an experiment in which 0.092 mgm. of adrenalin was given 1 minute before the start. The larger amount of adrenalin was an overdose; after 50 minutes of exercise the animal became much excited and refused to run further although the rate was only

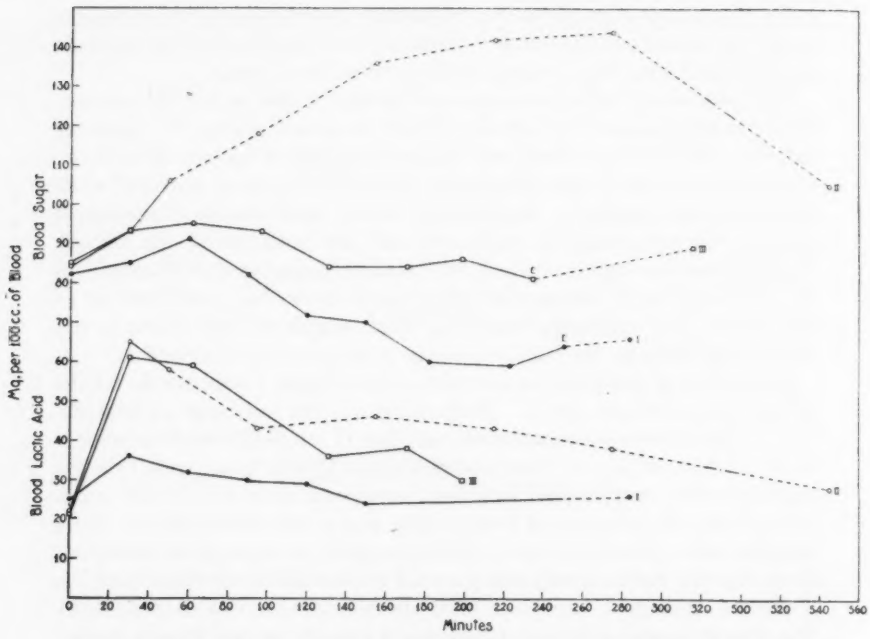

Fig. 3. Graphs showing changes in the level of blood sugar (upper group) and lactic acid (lower group) during work to exhaustion (solid lines) and after rest (dotted lines) in the same animal (dog B) under different conditions. Each mark in a line indicates an analysis. For further description see text.

109 m. per minute. The blood sugar rose to 106 mgm. per cent during the exercise, and 3 hours and 45 minutes later had reached a value of 144. The lactic acid rose to 65 mgm. per cent in the first 30 minutes of exercise whereas in the normal it reached a value of 36 mgm. only. The smaller dose of adrenalin also induced a rise of the lactic acid level above the normal rise and also an unusual rise of blood sugar, but these effects were less marked than with the larger dose. Similar results were obtained on dog D. A dose of 0.066 mgm. of adrenalin before the start raised the lactic acid in the

blood to 70 mgm. per cent and kept the blood sugar over 90 mgm. per cent during the first hour of exercise instead of letting it drop to about 80 mgm. It is noteworthy that as with dog A so likewise with dogs B and D a preliminary moderate dose of adrenalin had no favorable effect on the capacity for prolonged labor and that exhaustion was not closely related to the level of either glucose or lactic acid in the blood.

The evidence that adrenalin has a favorable action in muscular fatigue (see Gruber, 1919) and the absence of such action in the experiments just

TABLE 3

Details of two experiments on dog G, illustrating how adrenalin action is tested on a fatigued animal, and showing the consequent increased capacity to continue running

	EXPERIMENT III	
	Before adrenalin	After adrenalin
Date	3-27-27	
Rate (m./min.)	126	128
Adrenalin (mgm./kg.)		0.02 0.02
Work (min.)	78 16 7 10 2 = 113	32 14 = 46
Rest (min.)	6 6 7 5	8 8
Performance	S S I I R	S S
Condition at end	E	T
Energy (100 kgm.)	16	7
Per cent incr. energy		44

	EXPERIMENT IV	
	Before adrenalin	After adrenalin
Date	3-29-27	
Rate (m./min.)	125	132
Adrenalin (mgm./kg.)		0.02 0.02
Work (min.)	81 30 62 8 5 2 = 188	41 21 = 62
Rest (min.)	5 9 6 6 6	6 6
Performance	S S S I R R	S S
Condition at end	E	F
Energy (1000 kgm.)	26	9
Per cent incr. energy		35

described led us to consider whether it had not failed because it was injected before running started and, having transient efficacy, could not be influential when fatigue became prominent. Furthermore it was clear that a seemingly small dose (0.17 mgm. per kg. of body weight) can exert a harmful rather than a beneficial influence. Accordingly we decided to test the effect on animals already fatigued, and, if positive results were indicated, to learn a favorable dosage. The animals, therefore, were first exhausted as described above, and then, while they were in the exhausted state (in some

instances refusing to stand), an injection of 0.02 to 0.04 mgm. per kg. was given, and occasionally repeated, and the animal again exercised. The detailed results of two such experiments are recorded in table 3. The effect of the small dose of adrenalin was most striking. The animal, almost completely exhausted and refusing to run for more than 2 minutes without rest,

TABLE 4

Records of eight pairs of tests on five animals, showing the increased working capacity after adrenalin injections

In the first test of each pair the animal ran until exhausted; adrenalin was then given and the animal at once began to run again and continued until again exhausted or nearly so.

ANIMAL	EXPERIMENT NUMBER AND DATE	ADRENALIN	WORK	RATE	PERFORMANCE	CONDITION AT END	ENERGY (1000 KGM.)	PER CENT INCR. ENERGY
		mgm./kg.	*minutes*	*m./minute*				
G	III		113	126	S-I-I	E	16	
	3-27-27	0.02, 0.02	46	128	S-S-S	T	7	44
	IV		188	125	S-S-R	E	26	
	3-29-27	0.02, 0.02	62	132	S-S-S	F	9	35
H	I		66	172	S-S-R	E	13	
	4- 5-27	0.02, 0.02	22	127	S-I-R	E	3	23
	II		75	206	S-S-I	E	17	
	4- 7-27	0.02,0.02,0.02	21	262	S-S-S	E	6	35
K	II		47	124	V-V-I	E	7	
	11- 3-27	0.04						*
	III		114	124	V-S-I	E	16	
	11- 5-27	0.02, 0.02	24	124	S-S-R	E	3	19
L	III		167	124	V-V-R	E	23	
	11-10-27	0.02, 0.02	26	124	V-V-R	E	4	17
M	III		100	124	V-V-V	E	14	
	12- 8-27	0.02, 0.02	20	124	S-S-S	T	3	21

* Animal became excited and refused to run.

ran steadily without stopping for 32 and 41 minutes respectively in the two experiments, and at a faster pace than in the earlier periods. Apparently incapable of any further work the dog continued after the injection and exhibited an increase of energy amounting to 44 per cent in one test and 35 per cent in the other.

In table 4 is a summary of eight experiments on five different animals, similar to the two reported in table 3 and including them. In one test on dog K, experiment II, adrenalin in a dose of 0.04 mgm. per kg. of body weight produced in the fatigued animal effects similar to those seen in dog B when 0.174 mgm. per kg. was injected before the start of the exercise (see p. 688). An injection of 0.02 mgm. per kg. was found to be favorable and had the advantage that it could be repeated without producing the effects of an overdose. With this amount of adrenalin administered and repeated once or twice the exhausted animals showed a remarkable renewal of their ability to run. Not infrequently their performance was more uniform and consistent than in the first period, and in some instances their second period ended with the animal "tired" or "fatigued" rather than "exhausted." Further, the pace in the second period was with one exception either as great as or greater than the pace in the first period. As the last column of table 4 shows, the energy output in the seven completed tests was increased by use of adrenalin between 17 and 44 per cent.

Effects of inactivating the adrenal glands. Such striking influence of injected adrenalin as that just described led us to suppose that it would make considerable difference in ability to work if the adrenal glands were rendered inactive. That this anticipation was not realized has already been shown by the results reported in figure 2 (dog A). Although training might be expected to increase the endurance of the animal, it was not at all expected that after removal of one adrenal and denervation of the other the performance would be considerably better than before and that even after demedullation of the remaining gland the energy output would be as great as under standard conditions.

The observations on dog A, however, were confirmed by the tests of dog L. As shown in table 4, on November 10, 1927, this animal ran in the main vigorously for 167 minutes, and refused to run only after it had put forth 23,000 kilogrammeters of energy. On November 15, the right adrenal gland was extirpated and the left denervated. A month later (Dec. 15), the dog ran at the same rate as before 179 minutes and put forth 25,000 kilogrammeters. It is clear that adrenal inactivation did not lessen the capability of long continued labor. As shown in figure 2, whereas inactivation of the adrenals had no noteworthy influence on the glucose, it resulted in a *smaller increase of the lactic acid percentage in the blood during vigorous work.* In the experiment there represented this effect was associated with a longer period of work than usual. It is improbable that a causal relation exists between these conditions, for reasons already given (see p. 687), and also because adrenalin increases both the working capacity and the concentration of lactic acid in the blood.

The highly favorable results of adrenalin injections and the negative effects of eliminating the adrenal medulla from service present a discrepancy

which calls for explanation. We regret that we have no definite and decisive facts to bring to bear on the questions raised. It is possible that sympathetic impulses play a compensatory rôle to some degree, and that in the absence of secreted adrenin they are adequate for the ordinary grind of a long run. The secretion of adrenin under such circumstances is moderate (see Cannon and Britton, 1927). A physiological substitute for injected adrenalin, which might show the same striking results, would be an emotional discharge *when fatigue is well marked.* Cases of dissipation of the state of fatigue both in lower animals and in man by deep emotional disturbance have been reported to us. We have attempted to rouse fatigued dogs by bringing a cat before them, but without successfully producing excitement. It is possible, therefore, that the reason there is little difference between the performance of the animal when normal and when deprived of the service of the adrenal apparatus is that the normal animal does not use that apparatus to any noteworthy degree in routine running, but only in unusual emergencies.

Effect of cutting sympathetic nerves to skeletal muscles. The recent interest in the possible function of sympathetic nerve filaments distributed to skeletal muscles, especially in relation to fatigue (see Orbeli, 1924; Wastl, 1925; Tower, 1926), together with our present tests of endurance, made significant an observation on dog L. As stated above (p. 691) this animal ran 167 minutes at the rate of 124 meters per minute when trained; he ran 179 minutes at the same rate after adrenal inactivation. On December 20, 1927, the lower abdominal sympathetic strands were removed from near the kidneys to beyond the pelvic brim. The hind limbs were thus deprived of their sympathetic innervation. On January 5, 1928, this dog ran 166 minutes at the same rate as before, putting forth 23,000 kilogrammeters of energy, before exhaustion caused him to stop. There was no indication that his performance was checked by failure of muscles deprived of sympathetic fibers. This observation was confirmed on dog D whose stellate ganglia were removed on April 7, 1926. A few days before the operation the animal ran for 110 minutes at the rate of 107 meters per minute and at the end was "exhausted." On May 11—thirty-four days after the operation—he ran 107 minutes at a slightly slower rate (100 meters per minute), to be sure, but at the end was only "fatigued." The destruction of the sympathetic nerve supply to the forelimbs had no marked depressive influence on the endurance of the animal.

Effect of cutting the hepatic nerves. Because of the discharge of a substance from the liver which accelerates the heart (Cannon and Uridil, 1921), one of the first experiments we tried was to see whether section of the liver nerves would affect the working capacity. Dog B was trained until a standard was established,—a high one, in this instance, at 235 to 251 minutes of running before exhaustion. Then, on May 26, 1926, the liver

was denervated and the adrenals inactivated. Thereupon, although the animal was in good condition, the duration of running was never equal to the standard—eighteen days after the operation the record was 94 minutes at 121 meters per minute instead of 251 minutes at that rate (see table 5).

TABLE 5

Records showing decrease of working capacity after denervation of the liver, and restoration by adrenalin

ANIMAL	EXPERIMENT NUMBER	DATE	WORK	RATE	PERFORMANCE	CONDITION AT END	ENERGY (1000 KGM.)	
			minutes	*m./minutes*				
B	V	3- 5-26	251	121	S-I-R	E	34	
	VI	4- 8-26	235	115	S-I-R	E	30	5-26-26, liver denervated, adrenals inactivated
	VII	6- 2-26	84	144	I-I-R	E	14	
	IX	6-10-26	165	114	I-S-R	F	21	Adrenalin (0.025 mgm./kg.) at start, twice repeated
	X	6-13-26	94	121	I-R-R	E	13	
	XIV	10 -1-26	84	171	I-R-R	F	16	
E	III	11-11-26	145	122	S-V-R	F	20	11-16-26, nerves cut on duodeno-hepatic artery, good recovery
	V	11-26-26	60	116	S-I-R	E	8	
	VI	11-29-26	105	118	V-I-R	E	14	Adrenalin (0.02 mgm./kg.) at start, repeated later
	IX	1-13-27	55	111	I-I-R	F	7	1-20-27, adrenals inactivated, good recovery
	XII	1-31-27	57	117	R-I-I	E	7	
	XIII	2- 4-27	157	127	S-S-I	T	22	Adrenalin (0.02 mgm./kg.) at start, twice repeated
	XIV	2- 7-27	39	121	S-R-R	F	5	
K	III	11- 5-27	114	124	V-S-R	E	16	
			24	124	S-S-R	E	3	Adrenalin (0.02 mgm./kg.) twice. Cf. table 4
	IV	11- 6-27	110	124	V-V-I	E	15	11- 8-27, nerves cut on hepatic artery
	VIII	12-10-27	52	124	V-I-R	E	7	
	IX	12-12-27	50	124	V-V-R	E	7	
			20	124	I-V-I	F	3	Adrenalin (0.04 mgm./kg.) twice

In this experiment, however, the adrenal inactivation complicated the procedure.

Though other evidence indicated that excluding the influence of the adrenal medulla did not lessen the working time, nevertheless it was de-

sirable to test the effect of hepatic denervation with adrenals intact. Dog E ran 145 minutes at the rate of 122 meters per minute on November 11, 1926. On November 16 the nerves on the duodeno-hepatic artery were cut; ten days later and again more than two months later the running time was 60 and 55 minutes respectively in spite of slower speed (116 and 111 m. per min.). On January 20, 1927, the adrenals were inactivated; the operations made no marked change in the endurance of the animal (see table 5).

There was still another possibility—i.e., that in cutting the nerves on the duodeno-hepatic artery, the head of the pancreas, as well as the liver, was deprived of important nervous influences. To learn whether pancreatic denervation played a rôle, the nerves on the hepatic artery were destroyed in dog K on November 8, 1927, and the fibers to the pancreas left. Runs for 114 and 110 minutes at 124 meters per minute were thereby reduced, after more than a month had been allowed for recovery, to 52 and 50 minutes at the same rate (see table 5). It should be emphasized that in this and in the other cases the animals had been gradually trained to run again after the operation, and that the figures given represent maximal capability.

Altogether the results reported in table 5 show that section of the liver nerves, quite apart from denervation of the adrenals and pancreas, results in a marked decrease of the ability of the animal to run. This effect cannot be attributed to the abdominal operation itself, for, as shown in the foregoing section, when an equally serious operation has been performed—the right adrenal gland extirpated, the upper abdominal sympathetic strands removed, and the left splanchnic nerves severed—the workng capacity is not reduced.

As shown in table 5, the effect of adrenalin injections was tried on the animals with the hepatic nerves cut. The injections were given repeatedly, as indicated, commonly at the start and again when signs of exhaustion had appeared. In every instance there was a striking increase in the running time and in the energy output. Dog B, on June 13, ran 94 minutes and put forth 13,000 kgm. of energy. Three days before, when adrenalin was injected in repeated doses of 0.02 mgm. per kg., the time was 165 minutes and the energy 21,000 kgm. Unfortunately in this comparative test the speed was slower (114 m. per min. instead of 121) when adrenalin was used. Similar results, however, were obtained in more critical experiments on the other animals. Dog E ran 60 minutes at the rate of 116 m. per minute on November 26, 1926, and 55 minutes at 111 m. per minute on January 13, 1927. When adrenalin was administered on November 29, 1926, he ran nearly twice as long and at a faster pace (105 minutes at the rate of 118 m. per min.). In the records of dog K the additional running time is shown after adrenalin was injected; an increased performance occurred (Dec. 12, 1927) amounting to 40 per cent. It is noteworthy that in this experiment the dog tolerated two doses of adrenalin of 0.04 mgm. per kg. each, whereas before

the hepatic nerves were cut a single dose of that amount proved too great (cf. table 4); it is noteworthy also that the percentage increase of work, following the injections, was nearly twice what it was before the operation (cf. Nov. 5, and Dec. 12, 1927, table 5).

The faster heart rate in muscular exercise, the possible absence of a cardio-accelerator substance after hepatic denervation, and the helpful action of adrenalin (which might act favorably on the heart both directly and indirectly *via* the liver) led to an inspection of the records to learn the effects on the cardiac rate of section of the liver nerves. As a rule the maximal heart rate after denervation of the liver and inactivation of the adrenals is

TABLE 6

Average maximal heart rates of dogs, resting and working, while normal, after denervation of the liver (with adrenals inactivated), and when thereupon adrenalin was administered

DOG	NUMBER OF EXPERIMENTS	BEFORE OPERATION		AFTER OPERATION			
				Before adrenalin		After adrenalin	
		Resting	Working	Resting	Working	Resting	Working
A	7	95	172				
	4			90	147		
	3					90	157
B	6	81	162				
	6			70	137		
	4					69	147
C	3	95	160				
	3			88	144		
	2					89	150
D	4	89	171				
	3			100	144		
	3					98	152

lower than it was before. The average reduction of the maximal rates in four animals is shown in table 6. This was suggesive as a possible explanation of the decreased capacity of the animals. The faster rate during work when, after operation, adrenalin was given seemed to fit the idea that somehow failure of the heart to speed up was accountable for the poor performance after section of the adrenal and hepatic denervation. In some individual experiments, however, that explanation does not prove adequate. Dog E (table 5), for example, had on November 11, 1926 a basal heart rate of 72, which rose to 120 during work. After section of the liver nerves, the maximal rate during work was 144 (Nov. 26) with much less ability to run.

On November 29, when adrenalin was given, the performance was much improved, but it was not due to a faster heart rate—the maximal rate was 120 beats per minute. Also on January 13, without adrenalin, the maximal rate was 156 beats per minute, and the energy output 7,000 kgm. before exhaustion. When adrenalin was given at the start and repeated once later (on Jan. 18—not recorded in table 5), the energy output was 12,000 kgm. before exhaustion, and the maximal heart rate was again 156 beats. Furthermore, in normal animals the longer period of running and the greater output of energy following the injection of adrenalin (cf. table 4) was often associated with no higher heart rate and might be associated with less rise. For example, the maximal heart rate of dog G in *both parts* of experiment III (table 4) was 168 beats per minute, and in both parts of experiment IV it was 192—i.e., adrenalin did not raise the maximal rate. And in experiments I and II of dog H the rate was 168 in the fore period and 144 during the further run under the influence of adrenalin. Although this evidence indicates that adrenalin plays a rôle apart from increasing the heart rate, it does not exclude the possibility of its being a beneficial agent in favoring the action of cardiac muscle in many cases after the liver nerves have been cut.

Effects of cardiac denervation and subsequent adrenalin injections on the capacity to work. The experiment most frequently mentioned to prove the importance of the cardiac nerves in adapting the heart rate to muscular work was that of Friedenthal (1902). He cut the right vagus below the recurrent laryngeal branch, destroyed the middle and lower roots of the left vago-accessory nerves at the medulla, removed the lower cervical and upper thoracic sympathetic chain,—and supposed that he had denervated the heart. Evidence has been accumulating to show that accelerator nerves pass to the heart from the mid-thoracic region not only in the cat (Cannon, Lewis and Britton, 1926), but also possibly in the dog (Ionescu and Enachescu, 1928). These Friedenthal did not know about. He did not record the heart rate in his animal. He did not realize that even with the heart denervated it could be accelerated by secreted adrenin. For all these reasons it seems probable that Friedenthal's dog was not a thoroughly satisfactory animal on which to test the importance of cardiac adaptation.

In dog A of our series the heart was completely denervated by the method devised by experiments on the cat (see Cannon, Lewis and Britton, 1926). The liver nerves had previously been cut, the right adrenal removed and the left adrenal medulla sucked out; all the known abdominal humoral agencies were thus excluded from action. Before the cardiac denervation the dog ran (Sept. 24, 1926) 167 minutes at 110 m. per minute with an energy output of 20,000 kgm. and with the pulse rising from 96 to 156 beats per minute. On October 11 she ran, under the influence of adrenalin, 203 minutes at 148 m. per minute, with an energy output of 33,000 kgm. and with the pulse rising

from 84 to 156. The heart was denervated on November 1. Thereafter the greatest increase ever observed when the animal was induced to run was 12 beats per minute. The animal could not be made to run to an extent resulting in signs of being tired. She would travel for a relatively short time and then would brace all four feet and refuse to move. Administration of adrenalin, however, would raise the heart rate from its basal (96 beats per min.) to 120 and therewith considerably augment the work capacity. For example, on December 1, the animal ran in short periods (two of 13 and 15 minutes, others of 2 or 3 minutes only) for 44 minutes at 129 m. per minute—an energy output of 6,000 kgm. No further work would the animal perform. The heart rate remained at 96. Four days later (Dec. 4) the animal was given adrenalin (0.035 mgm. per kg.) at the start and twice later. She ran for an hour and a half the first half hour "steadily"—at 123 m. per minute, putting forth 12,000 kgm. of energy. The heart rate was raised from 96 to 120. These results were typical. They show quite strikingly the great limitation of the ability to run when the heart does not take part in the organic adjustments, and the added ability when the pulse is accelerated.

Discussion. The outstanding facts in the foregoing pages appear to be the absence of close relations between the levels of glucose and lactic acid in the blood and the duration of the work, the diminished running capacity after the liver was denervated, and the beneficial effects of adrenalin after exhaustion has appeared. And the central question arises,—what is the cause of the exhaustion?

The most natural and simple explanation of that lessening or failure of performance which characterizes fatigue is a consumption of the energy-yielding material or an accumulation of waste in the active structures.

The fall of blood sugar in muscular exercise has been repeatedly noted (see Mangus-Levy, 1925). Observations on Marathon runners have been reported as showing a close correlation between the condition of the runner and the glycemic level (Levine, Gordon and Derick, 1924). The glucose level in the blood may not be a good measure of the glucose content of the tissues. After a meal rich in sugar the glycemic level in the blood may be lower than usual (Folin and Berglund, 1922), and in prolonged muscular effort the local supply in the active structures may be reduced to a degree not more than roughly indicated by the percentage in the circulation. The experiments described above yield, of course, no direct information regarding the conditions in the laboring muscles. They do show, however, that exhaustion can occur when the glycemic level is high, whether in consequence of natural processes or in consequence of injected glucose or adrenalin. The running, therefore, need not have stopped because of failure of the glucose supply—an abundance may be at hand when the animal is too greatly fatigued to be able to continue work.

The evidence that muscles utilize not only carbohydrate but other food stuffs in doing work (see Meyerhof and Himwich, 1924; Rapport and Ralli, 1928), and the consideration that not all the available energy-yielding material could be used up in such tests as animals were subjected to in our experiments, and, further, that "exhausted" animals were made capable of much further labor by injections of adrenalin—all three points support the conclusion that inability to continue running is not due to lack of "fuel."

Lactic acid is the waste product of muscular exercise which is most commonly associated with reduced efficiency. The ability of ventricular muscle to develop tension is closely and inversely correlated with its content of lactic acid (Redfield and Medearis, 1926). Indeed, muscular fatigue has been defined as a state in which lactic acid is produced faster than it can be removed by neutralization or oxidation. These views are based upon studies of isolated muscles. There is no doubt that the animals in the present research reached the limit of their ability to work. There is evidence that the lactic acid concentration in muscles is probably close to that in blood: 1, with very strenuous exercise the concentration in the blood may be greatly increased (Barr, Himwich and Green, 1923; Hill, Long and Lupton, 1924); 2, with moderate exercise the lactic acid content of working muscles *in situ* is not significantly higher than that of resting muscles or of blood (Embden, Schmitz and Meinke, 1922); and 3, lactic acid formed in especially hard-worked muscles is quickly washed away by the blood and taken up by the less active muscles and the liver (Barr and Himwich, 1923). In our experiments the concentration of lactic acid in the blood was highest early in the exercise when the animals were commonly running at a steady pace; it was usually much lower when the state of exhaustion was reached; it was low during work after inactivation of the adrenals without any marked change in performance; and administration of adrenalin, if influential, increases the lactic acid in the blood to an unusual degree and yet improves the ability to run. These considerations make difficult any conclusion regarding the influence of lactic acid in the blood on the limitation of working ability. It seems that the exhaustion which checks the doing of further work does not result from a piling up of lactic acid; certainly not in the blood and probably not in the active tissues.

The action of adrenalin in restoring the ability of the exhausted animal to go on working was remarkable. The full story of the way in which adrenalin affects fatigued muscle is not yet known. Physiological amounts of adrenalin injected intravenously are capable of restoring, suddenly, the lost responsiveness of fatigued muscle (Gruber, 1914); adrenalin shortens the latent and contraction periods of fatigued muscle and increases the height of the contraction (Gruber, 1924); it can bring back to a muscle an hour or more after death of the organism the contractile function (Gruber and Fellows, 1918); and unpublished experiments performed in this labora-

tory by H. B. Hunt indicate that it will allow the heart muscle to continue beating after being worked to a standstill in an atmosphere of nitrogen. It is clear that adrenalin has effects on muscular action quite apart from mobilizing sugar in the blood or improving the circulation. In our experiments the injected adrenalin may have exerted its favorable influence in all three ways—by glycogenolysis, by bettering the blood flow and by local action of a character not yet determined.

The striking reduction of the working ability which followed section of the liver nerves requires comment. That operation, especially when associated with adrenal inactivation, might be expected to lower the glycemic level to a damaging degree. That proved not to be true. The glycemic level and the course of the glycemic changes during exercise were not altered to any noteworthy degree either in dog E (with liver alone denervated) or in dog B (with liver denervated and adrenals inactivated).

It seems probable, also, that the depressant effect of cutting the hepatic nerves was not due to an unfavorable influence therefrom on removal of lactic acid from active tissues. To be sure, section of the liver nerves (in addition to adrenal inactivation) in dogs B and D was followed by a smaller concentration of lactic acid in the blood. Further, when adrenalin was administered to such animals the lactic acid was increased in the blood in the usual way during exercise, and the ability to run was prolonged. Is it not possible that adrenalin improved the working capacity by bringing forth lactic acid from the tissues where it was accumulating and consequently hampering contraction? The fact that functional exclusion of the adrenals alone is attended by reduced percentage of lactic acid and by no reduction of the endurance of the animals (see dogs A and L, pp. 687 and 691) throws doubt on that suggestion.

The foregoing considerations support the argument already advanced that glucose and lactic acid in the blood are not of primary concern as limiting factors in muscular work. They do not throw light, however, on the damaging results of isolating the liver from the central nervous system. Why that operation should affect muscular performance so markedly is not at all clear. The liver, when stimulated through its nerves, gives off a substance which has an excitatory effect on the contractions of the heart (Cannon and Uridil, 1921). It is possible that this unknown substance has a favorable action also on the contractions of other muscle, and that when the liver is denervated it fails to be discharged.

It must be admitted that this discussion—indeed, this research as a whole—probably raises more questions than it answers. We do not know the relations between lactic acid and glucose in blood and in tissues; we do not know about the effects of adrenalin on fatigued muscle; we do not know whether a substance produced by the liver influences the activity of skeletal muscle; we do not know the *rates* at which changes can occur in the body—

possibly of fundamental importance in explaining fatigue phenomena. Further we have little evidence as to what is occurring in the nerve cells on whose functioning the muscular contractions depends. It is a neuromuscular system with which we are concerned and we have dealt mainly with the peripheral aspects of it. Until we have further insight into these complex relations, and can answer some of the questions just raised, the central problem as to the cause of exhaustion must remain unsettled.

SUMMARY

By means of a treadmill, moving at a known speed and set at known inclination, various conditions which might affect the maximal capacity of dogs to run were studied.

With repetition of the tests the maximal ability increases (see tables 1 and 2).

The blood sugar as a rule decreases during the exercise period and rises slowly in the subsequent recovery period. No close relation was found between the glycemic level and the performance of the animal (see figs. 1 and 2).

Increase of the glucose percentage of the blood by preliminary injection of glucose or adrenalin has no favorable action on performance (see figs. 2 and 3).

The lactic acid in the blood rises during the first stages of work; then begins a decline which continues during the recovery period. No close relation was found between the percentage of lactic acid in the blood and the condition and performance of the animal (see fig. 1).

Preliminary injection of adrenalin may cause a rise of the lactic acid percentage higher than normal, but this is not associated with any definite effect on performance (see figs. 2 and 3).

A "large" dose of adrenalin (0.174 mgm. per kg.) induces excitement and lessens the capacity to run.

If animals run until exhausted and are then given subcutaneously "small" doses of adrenalin (0.02 mgm. per kg.), they recover to a striking degree their ability to run. They may put forth energy from 17 to 44 per cent additional to what they have already put forth (see tables 3 and 4).

Inactivation of the adrenal glands by removal of one and denervation of the other does not lessen the capacity for long-continued labor (see fig. 2).

The inactivation is associated with no marked influence on the glycemic level, but with a noteworthy reduction of the lactic acid rise during vigorous work (see fig. 2).

Severance of the sympathetic nerves of skeletal muscles does not reduce the maximal performance.

Severance of the hepatic nerves results in a marked decrease of the capacity to run. Reasons are given for not attributing this result to the operation

or to effects on the heart rate. The capacity to run is largely restored by injection of adrenalin (see table 5).

Denervation of the heart, the adrenals and the liver is followed by a striking reduction in the ability to run. Injection of adrenalin then accelerates the heart and greatly increases the working capacity.

The outstanding phenomena—the ineffectiveness of the glucose and lactic acid percentages in the blood, the favorable action of adrenalin, the unfavorable effect of hepatic denervation—are discussed in their bearings on the phenomena of exhaustion, but with questions suggested rather than answered.

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